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ASPECTS OF LEUCOCYTE AND FAT FILTRATION DURING CARDIAC SURGERY

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Contents

CHAPTER 1

INTRODUCTION

1. Means to achieve a reduction of the inflammatory response in cardiac surgical patients

The generalized inflammatory response that occurs after cardiac surgery, elicits a combined reaction from at least the immune system, the coagulation system and the endothelial cell system. Activated leucocytes play a key role in this process by their interaction with the endothelium, by their interaction with the cardiopulmonary bypass circuit, and by their role in reperfusion injury, where they form platelet-leucocyte complexes. To minimize or even prevent postoperative tissue injury, it is thus attractive to target the leucocytes. This has been tried with various methods for nearly all stages of the inflammatory pathway. Currently, the most important strategies focus either on the prevention of leucocyte activation, or on the modulation of the inflammatory response, or on ultrafiltration techniques.

Leucocyte activation may be prevented with heparin coated bypass circuits, which reduce contact activation and possess an enhanced biocompatibility. However, in a large European trial, clinical benefits could not be demonstrated in low-risk patients.[1] In high-risk patients, the use of a heparin coated bypass circuit reduced the time for postoperative ventilatory support and intensive care unit stay,[2] but this could not be confirmed in another study.[3]

Pharmacological agents may also prevent leucocyte activation. A serine protease inhibitor with a variety of actions, aprotinin, is frequently used. Aprotinin and prednisolone have been found to attenuate the generation of tumor necrosis factor and the upregulation of leucocyte adhesion molecules.[4] A meta-analysis showed that aprotinin, besides a reduction in blood use, reduced perioperative mortality.[5] However, two recently published studies could not demonstrate an anti-inflammatory effect of aprotinin,[6,7] although postoperative blood loss was reduced. Due to the cost and to the sensibilisation that occurs in 5% of the patients, aprotinin is generally reserved for redo-operations.

Pharmacological agents are also used to modify the inflammatory response. For this purpose corticosteroids, and particular dexamethasone, are often used. Corticosteroids probably change the cytokine balance from proinflammatory to anti-inflammatory.[8] Corticosteroids reduce leucocyte activation and pulmonary leucocyte sequestration.[4,9] Although dexamethasone has been shown to decrease the concentration of C-reactive protein on the first postoperative day, clear clinical benefits in terms of postoperative oxygenation, time on mechanical ventilation, or intensive care unit stay have not been demonstrated.[10-12] Use of dexamethasone may even be detrimental by delaying early postoperative tracheal extubation, and initiating postoperative hyperglycemia.[11] In addition, there is concern about the systemic effects, for example the associated immunosuppression.

A relative novel pharmacological agent is the phosphodiesterase inhibitor pentoxifylline, which has recently been used in a porcine model of lung transplantation.[13] In this animal study, the use of pentoxifylline was as effective as leucocyte depletion in preventing reperfusion injury. Several clinical studies are available in humans during cardiac surgery that also demonstrate an effect on pulmonary leucocyte sequestration after cardiopulmonary bypass.[14,15] However, whether pretreatment with pentoxifylline will improve outcome in patients remains to be elucidated. Pentoxifylline pretreatment in cardiac surgical patients attenuated a postoperative deterioration of endothelial, renal, and liver function, but in a patient

group at high risk of systemic inflammatory response after cardiac surgery supplemental pentoxifylline treatment did not reduce mortality.[16,17]

Ultrafiltration techniques are used to restore the intraoperative fluid balance and to reduce the inflammatory response. This approach is based on the idea that ultrafiltration removes factors that trigger the inflammatory response. Ultrafiltration has found a place mainly in paediatric cardiac surgery where it has been shown to reduce body water, and to increase the haematocrit.[18] In adults however, the effects are less clear and in a recently published study comprising 3,988 patients who underwent coronary artery bypass grafting, it was suggested that ultrafiltration may have adverse effects on operative outcome.[19]

Fat also contributes to postoperative tissue injury. Fat microemboli have been demonstrated in brain tissue after cardiopulmonary bypass.[20] These microemboli were related to the retransfusion of cardiotomy suction blood,[21] and were associated with postoperative neurocognitive dysfunction. In addition, the role of fat on tissue injury is underestimated, because fat microemboli have not only been demonstrated in brain tissue after cardiopulmonary bypass, but also in lung and renal tissue.[22,23]

Cell savers are increasingly used to process cardiotomy suction blood, but these devices might be less than ideal for several reasons. First, fat is not completely removed by cell savers. Second, their use is expensive and requires attention and time to process. Third, processed cell saver blood contains increased levels of interleukin-I and activated leucocytes, which may aggravate the inflammatory reaction associated with cardiopulmonary bypass.[24] Kaza et al. found that cell savers were not more effective than a filter after the cardiotomy reservoir for the elimination of small and large fat emboli.[25]

Therefore leucocyte and fat depletion by means of a filter may offer a good and practical alternative to modify the postoperative inflammatory response in cardiac surgical patients. The aim of this thesis is to demonstrate that leucocyte and fat filtration, applied in the setting of cardiac surgery, has a beneficial effect on inflammatory markers and postoperative organ injury.

2. *History of leucocyte filtration*

Various aspects of leucocytes as markers of infection were already known in the beginning of the 20th century. For example, Gibson, a surgeon, wrote in 1906 in the first clinical paper on leucocyte differential count that "... the differential blood count and its relation to the total leucocytosis is today the most valuable diagnostic and prognostic aid in acute surgical diseases that is furnished by any of the methods of blood examination."[26] In 1928, Fleming, a pathologist, was the first to use a cotton wool plug as a filter for the removal of leucocytes from blood.[27] His apparatus is shown in figure 1, and consists of a bend glass tube with a constriction. Cotton wool was introduced in the constricted limb of the tube and pressed down as tightly as possible with a cork-borer. Blood was placed above the cotton wool and under pressure forced through the cotton wool with a teat. The aim of Fleming was not the modification of the patient's inflammatory response, but he needed leucocyte depleted blood as a diluent for certain tests in connection with the antibacterial power of leucocytes. We demonstrate his apparatus, because the compressed cotton wool used in his filter resembles the structure of a modern depth filter and the pressure applied by the teat equals the pressure that may be generated to force blood through modern leucocyte depletion filters.

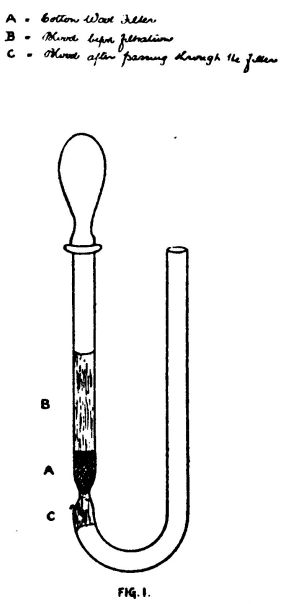

Figure 1. Leucocyte depletion filter as used by Fleming in 1928. A, compressed cotton wool; B, column of blood before the filter; C, Blood after passage through the filter

By the end of the second World War more was known about function and properties of leucocytes. It became clear that it was undesirable to have white blood cells in transfusion components. Thus, the history of leucocyte filtration has been closely related to the history of blood transfusion. From the beginning of transfusion medicine blood clots and debris were observed in transfusion bottles. This remained the case even after the introduction of citrated blood in 1914 by Hustin.[28] It was therefore necessary to filter the blood just before transfusion. This was initially accomplished by pouring the blood over gauze swabs, which was not only a cumbersome and messy undertaking, but also resulted in contamination of the blood. However, in 1939 several types of filter were in routine use, all allowing sterile processing of the blood.[29] These filters consisted of glass beads in a glass cylinder and were to be attached in line to the tubing of the transfusion system.

In the second World War stainless steel wire cloth incorporated in a glass cylinder was used.[30] Although these filters were intended to be disposable, their cost was high. They were therefore often cleaned after use, which was of course not easy. From then on the filtering of the blood became rapidly more sophisticated and less expensive, and from 1950, 230μm disposable filters were standard employed throughout the world.

In 1961 Swank accidentally made an important observation while studying blood viscosity in a model of small blood vessels. His observation served as a milestone for the rapid development of transfusion related filtration techniques, because it demonstrated the importance of leucocyte removal. He found, using a microfilter as model (figure 2), that very high pressures were necessary to force blood that was stored in acid-citrate-dextrose for 2 to 10 days through the filter. Microscopic examination of the filters revealed that many openings were occluded by debris and aggregates of platelets and leucocytes.[31] Swank then passed the old blood through a glass wool filter and found that after this procedure the pressure to force the blood

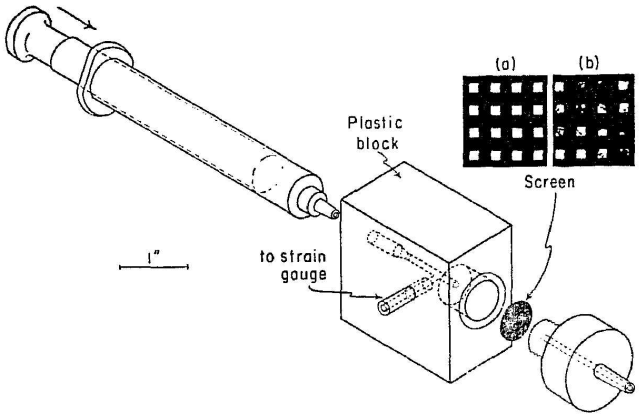

Figure 2: Microfilter as used by Swank in 1961. The plastic block is contained in a warming chamber. Inserts show a microfilter before (a) and after (b) testing of blood which required a high filtration pressure. The pressure is applied by the plunger of the syringe, as indicated by the arrow. Applied pressure is recorded by the strain gauge.

through his experimental micro-filter was similar to that of fresh blood. Most of the aggregates in the glass wool filter were less than 50 μm in size on microscopic examination and he called them micro-aggregates. A second observation was that plasma, that had been spun free of white blood cells and platelets did not require high pressure to force it through the microfilter, even after storage for 10 days. On the other hand, plasma containing leucocytes and platelets exhibited the same filtration problems after storage for 10 days as shown by whole blood. Dacron and polyester filters in wool form also removed the micro-aggregates effectively. In the late 1970s specific filters for routine leucocyte removal in clinical practice have been developed.

3a Leucocyte depletion filters

The first generation of leucocyte depletion filters for routine use that became available in the 1970s were made of cellulose and had a leucocyte removal rate of about 98%. They were developed for blood bank use to obtain leucocyte depleted blood for specific purposes such as the prevention of non-haemolytic transfusion reactions and viral transmission. However, these filters appeared also to activate complement C3, which promotes vasoconstriction and increases capillary permeability.[32] A second drawback was that the efficacy of leucocyte removal was determined by the flow over the filter. Filtration was therefore a slow process and took about 30 minutes for one unit of red blood cells.

Over the last years however, a new generation of filters has become available which combines rapid flow with an excellent leucocyte removal rate. These new filters remove 99,995% of the leucocytes from the blood, but for cardiopulmonary bypass perfusate this is somewhat lower with a 96.8% removal of leucocytes.[33] The improved flow properties allow them to be used in settings with higher fluid requirements. For an explanation of these improved flow properties, some aspects of the biomaterials and design that are used will now be discussed.

The design of a leucocyte depletion filter is of course a compromise between several properties. At this moment depth and screen filters are used. In depth filters, the filter material has the form of compressed wool fibres. These filters are made of polyester or sometimes polyurethane, and promote adhesion of the leucocytes throughout the filter material. In contrast, screen filters consist of layers of woven polyester filter material. In this type of filter the leucocytes are bound to subsequent

layers of filter material. Most leucocytes are thus trapped at the outermost portion of the filter and this may increase the resistance over the filter.

The way leucocytes are trapped inside the filter influences filter efficacy and capacity. At least 4 active and passive mechanisms have been described (figure 3).[34] The most important mechanism is adhesion. The negatively charged leucocytes are attached to the filter material by Van der Waals- and electrostatic forces. It is, therefore, an active process from the side of the leucocytes. The advantage is that a larger pore size is possible in the filter with subsequent higher flow rates. Passive mechanisms of leucocyte entrapment are blocking, bridging and interception. By blocking, the leucocyte is trapped in a pore between two fibres. By bridging, 2 or more leucocytes form an aggregate in a pore between two fibres. By interception, leucocytes are mechanically trapped in the dead space around the fibres. All these mechanisms may occur together.

Thus, properties of the filter material like surface charge and hydrophilicity greatly determine the efficacy of the filter. Therefore, coating of the filter material is often used to improve the filter efficacy. A coating of methacrylate creates a more positive surface charge that results in a stronger bond with the negatively charged leucocytes.[35] Hydrophilicity is important for optimal contact between the leucocytes and the fibres and thus for the subsequent adhesion. This implies that optimal leucocyte depletion can only occur if the whole filter is exposed to blood, which means that de-airing before use must be carefully performed. Insufficient de-airing results in disturbances of optimal blood flow and thus in a reduction of filter efficacy.

Another effect of the physicochemical properties of the filter material is that leucocytes appear predominantly to stick to the crossing points of the filter fibres.[36] Thus, more crossing points increase the efficacy of the filter. More crossing points require thinner fibres. However, thinner fibres also lead to an increase in resistance and thus to flow reduction.

The filter capacity depends on the construction of the filter, i.e. the available surface and the thickness of the filter. A simple rule is that the log of the leucocyte reduction in the filter relates to the thickness of the filter material.[36] The current generation of leucocyte depletion filters may be pressurized up to 300 mmHg. This allows rapid transfusion in a clinical setting, but decreases the efficacy as it has been shown that a longer contact time of the leucocytes in the filter increases the filter efficacy.

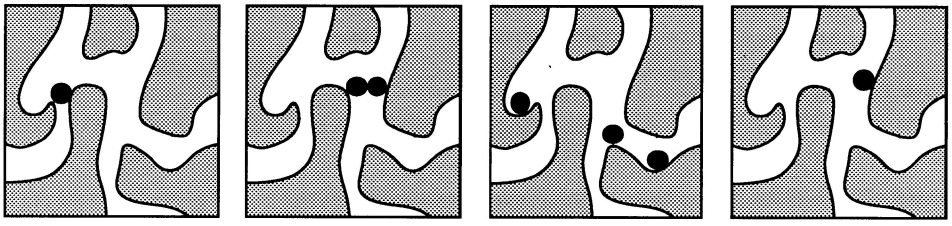

Figure 3. Diagram of proposed mechanisms of leucocyte retention in a depth filter according to Bruil. A, blocking; B, bridging; C, interception; D, adhesion. (Bruil A; Leucocyte filters. Thesis TU Twente; 1993)

The efficiency of the filters decreases over time as the filter becomes saturated with cells and debris.[37,38] For a blood cardioplegia filter this results in a pressure gradient of about 10mmHg at a mean flow of 300 ml.min^{-1}. In up to 2500 ml about 75% of the leucocytes were removed.[39]

One major drawback of the leucocyte depletion filters is the concomitant removal of platelets. Thus, application of leucocyte depletion filters may interfere with blood coagulation. This is a problem of all leucocyte depletion filters as generally 40% of the platelets that pass through the filter are trapped.[40] Allen et al. demonstrated a significant difference in platelet counts by drawing samples simultaneous up- and downstream of the filter.[41] Nonetheless, a certain platelet deposition on the polyester fibres of the filter facilitates the adherence of the leucocytes.[42] It appears that platelets have a higher affinity for the filter material than leucocytes,[43] while platelets have active surface receptors so that they rapidly establish a strong bond with leucocytes.[44] Another aspect of the removal of platelets is that platelets also are responsible for the release of different vasoactive substances. Therefore, removal of platelets could also lead to a reduction in thromboxane release and therefore to a reduction in vasoconstriction.[45]

3b. Fat removal filters

Little is known about the clinical filtration of fat emboli. As a consequence, part of the technology for fat filtration is derived from the diary food industry and water cleaning processes. For example, hydrophobic cotton fibres, which are obtained by acylation of cellulose, have a high selective affinity for fat in an aqueous medium.[46] They are used for water processing, but resemble the first generation of leucocyte depletion filters. Fat filtration appears to be technically difficult, because the process is temperature dependent. A fat removal filter removed 50% of the fat load at 37^{o}C which increased to 80% at 10^{o}C, due to the increased viscosity of the fat. However, at lower temperatures, haemolysis of the blood and clogging of the filter were observed.[47]

A standard polyester arterial line filter with a 40 μm pore size does not seem to filter fat during cardiopulmonary bypass.[48] However, passing cardiotomy suction blood through a standard 30 μm cardiotomy suction filter and subsequently through a 21 μm arterial line filter almost completely eliminated fat.[25] These findings suggest that the filter pore size is a major determinant for fat filtration. In 1973, Arrants et al. used an ordinary blood filter with a pore size of 35-40 μm, but they could not demonstrate a clinical effect.[23] Thus, a small pore size is necessary and supports the concept that fat globules are highly deformable.

Based on the work of Swank, Hill et al. used a dacron wool filter in the cardiotomy suction line.[49] This resulted in a reduction in postoperative cerebral dysfunction. Clark et al. studied the fat filtration characteristics of a packed polyester wool filter.[50] They found that significant quantities of solids, two thirds of which were fat, were removed by the filters. Their findings about the efficacy of polyester for the removal of fat are in line with a more recent study evaluating a specific fat removal filter.[51] This filter is also made from polyester fibers, but contains less filter material to improve the flow properties than the leucocyte removal filter from which it was derived. However, a specific coating of the filter material should compensate for this. In a laboratory study with reconstituted outdated blood and soya oil, the filter removed fat, but was less effective than a leucocyte depletion filter.[51]

This fat removal filter was also used in conjunction with a leucocyte removal filter for processed cell saver blood.[52] This combination again emphasizes the small pore size that is needed for fat filtration. However, a small pore size reduces the flow properties of the filter. Better coating of the filter fibers may offer an alternative. Therefore, sorbent technology which is currently used in dialysis filters, may hold promise for the future as a result of improved coating techniques.[53]

4. What is the evidence that leucocyte and fat depletion filters are beneficial during cardiac surgery?

In the 1980s attention was focussed on what happened during ischaemia and reperfusion in organs. Engler et al. demonstrated in dogs that the myocardial stunning which was observed after occlusion of the left anterior descending coronary artery up to 5 hours, resulted largely from reperfusion injury. They observed during reperfusion an incomplete restoration of the blood flow in the microvasculature of the heart. This so called no-reflow phenomenon was associated with capillary leucocyte plugging and endothelial cell protrusion and was based on an acute inflammatory response.[54] This revealed a central role for leucocytes and Engler et al. hypothesized that leucocyte depletion might be beneficial in the setting of ischaemia and reperfusion. They tested this hypothesis in their dog model and found that reperfusion with leucocyte depleted blood almost completely prevented reperfusion injury. In addition, leucocyte depletion prevented the increases in tissue water content seen in control hearts and decreased the incidence of ventricular arrhythmias.[55] Shortly after these observations, Kutsumi et al. applied leucocyte filtration clinically in the setting of ischaemia and reperfusion. After percutaneous transluminal coronary angioplasty (PTCA) they drew blood from the femoral vein which they passed through a leucocyte filter before it was injected in the coronary vessels. This procedure resulted in a significant reduction in reperfusion arrythmias.[56]

These findings on reperfusion injury stimulated research in the setting of heart and lung transplantation. Here, the beneficial effects of leucocyte depletion were quickly recognized and gradually found a clinical application during lung transplantation,[57] and as an adjunct to blood cardioplegia in the setting of cardiac transplantation.[58] Preliminary studies in dogs after cardiac transplantation indicated that reperfusion with leucocyte depleted blood increased stroke work and cardiac output,[58,59] and were soon followed by clinical studies that showed minimal histological changes and lower myocardial creatinine phosphokinase levels after reperfusion with leucocyte depleted blood.[60] However, clinical effects in terms of cardiac function were less clear.

As a result of the effects of leucocyte depleted reperfusion during cardiac transplantation, blood cardioplegia filters were developed. Only the target organ is depleted, while total body and side effects are minimal. This stimulated research in the setting of ischaemia and reperfusion and clinical studies were extended to emergency cardiac operations in patients who developed an acute myocardial infarction. Sawa et al. applied leucocyte depleted blood cardioplegia in elective and emergency patients.[61] The results of leucocyte depletion were better in emergency patients than in elective patients. Lower peak myocardial creatinine phosphokinase levels were measured in the emergency patients and less dopamine was required at weaning off cardiopulmonary bypass. This study was extended to patients with left ventricular hypertrophy, defined as a left ventricle mass >300 g, to investigate if

reperfusion injury was attenuated by the application of a leucocyte depletion filter for blood cardioplegia that was administered for the first 10 minutes after aortic cross clamp release.[62] Left ventricular biopsies had significantly lower scores for myocyte damage and for endothelial cell damage of capillaries in the leucocyte depleted group. The leucocyte depleted group also had lower myocardial creatinine phosphokinase levels, and needed less dopamine for weaning off cardiopulmonary bypass. No side effects of leucocyte depletion were noted. In a subsequent study the patients with left ventricular hypertrophy were divided in a group with a long and in a group with a short aortic cross clamp time.[63] The effects of leucocyte depletion were more pronounced in the group with the longer cross clamp times. Roth et al. also studied patients with depressed left ventricular function, using serial leucocyte depletion filters in the blood cardioplegia line.[64] Less dopamin was needed in their filter group, that also showed an increased left ventricular ejection fraction. Another interesting thing in this study was the use of two blood cardioplegia filters in line. The authors wanted to achieve a high degree of leucocyte depletion and felt that the documented efficacy of a standard blood cardioplegia filter was too low.[64] We also found that the efficacy of a leucocyte depletion filter was lower if cardiopulmonary bypass perfusate was used.[33]

These findings indicate that leucocyte depletion is more beneficial in patients that are clinically in less favourable circumstances. It also indicates that the systemic effects of leucocyte filtration are minimal.

A word of caution should be made with regard to the proper interpretation of the clinical results in the earlier studies. At that time only transfusion filters and cell separator technology were used for leucocyte depletion. The efficacy of these technologies is less than the efficacy of the current generation leucocyte depletion filters. Thus, the clinical effects may have been underestimated in the past.

Another important question that had to be resolved was how long leucocyte depletion should be performed in order to prevent reperfusion injury. This question was addressed by Breda et al.[65] They studied lung preservation in a rabbit model, and found that reperfusion with leucocyte depleted blood preserved lung function. Then they added again leucocytes to the perfusate and found that the addition of leucocytes after one hour of reperfusion did not cause significant injury to the lung.

In contrast with the findings in isolated organ perfusion, Bando et al. were the first to report a favourable systemic effect of leucocyte filtration.[66] They found in dogs, subjected to cardiopulmonary bypass, a reduction of free radicals and a preservation of pulmonary function by the application of a leucocyte depletion filter in the cardiopulmonary bypass circuit. These findings were repeated by Johnson et al. who also studied dogs during cardiopulmonary bypass, using a bubble oxygenator.[67] They found an improvement in pulmonary shunt in the filter group. In addition, histological investigation of the lungs after cardiopulmonary bypass revealed lower oedema scores in the dogs that had leucocyte depletion, which resulted in an improved gas exchange. These two landmark studies served as a starting point regarding the effects of systemic leucocyte depletion.

Most studies on systemic leucocyte depletion during cardiac surgery agree on the fact that filtration reduces postoperative leucocyte counts.[37,67-69] However, there is disagreement about the clinical effects on pulmonary or cardiac function. Some studies reported a short term improvement in postoperative oxygenation. Palanzo et al. found an improved arterial blood oxygenation. With 100% oxygen, patients in the

leucocyte depleted group had an arterial oxygen tension of 54.9 kPa vs. 46.4 kPa in the controls. The postoperative time on the ventilator was with 9.2 vs. 13.3 hours also shorter in the leucocyte depleted group than in the control group.[70] Johnson et al. reported a transient improved oxygenation in the leucocyte depleted group with a transient improved intrapulmonary shunt (19% filter vs. 24% controls), which was in agreement with their previous study on dogs.[71] Lust et al. also found a slight improvement in arterial blood oxygenation.[72] Others however, did not find an improved arterial blood oxygenation at all.[73] It should be noted that in all these studies arterial line filtration during the whole period of cardiopulmonary bypass was used.

Several factors may explain the reported differences in filter efficacy. Amongst them are the timing and duration of the filtration procedure during the operation and the type of filter used. For example, leucocyte depletion is commonly achieved with a filter in the arterial line throughout the cardiopulmonary bypass period.[37,70-74] However, using this procedure, high elastase levels have been demonstrated after the filter in the arterial line.[73] Elastase is a marker enzyme for leucocyte activation. Mair et al. found elevated systemic levels of elastase by the end of the filtration period,[74] and also Palanzo et al. could not demonstrate a reduction in systemic plasma elastase levels when applying leucocyte depletion in the arterial line of the cardiopulmonary bypass circuit.[70] These findings suggest that the leucocytes which were trapped in the arterial line filter become extensively activated. However, it may also be possible that activated leucocytes are preferentially trapped inside the filter. This was first suggested by Thurlow et al. when they investigated the expression of antigens on neutrophils using leucocyte-associated monoclonal antibodies.[75] In a subsequent small scale study in humans they concluded that the application of an arterial line filter did not result in a significant depletion of the leucocyte load during cardiopulmonary bypass, but that according to their indirect measurements of superoxide the activated forms of the leucocytes appeared to be depleted.[76]

The good results of leucocyte depletion during reperfusion, the moderate results of leucocyte depletion with an arterial line filter during the whole cardiopulmonary bypass period and the limited capacity of the filters, prompted Hachida et al. to remove the leucocytes from the circulation by a systemic filtration procedure in a restricted but well aimed time span.[77] They applied leucocyte depletion only in the reperfusion phase after aortic cross clamp release and reported an improved pulmonary index after 3 and 6 hours. Matheis et al. also applied leucocyte filtration in a short time period after aortic cross clamp release and found in the leucocyte depleted group a reduction in inotropic support and a reduction in troponin-T concentrations, indicating less myocardial damage.[78] However, Baksaas et al., using a similar procedure, found a reduction in circulating leucocyte counts, but could not demonstrate clinical differences with their control group.[79] They suggested that filtration during release of the aortic cross clamp was too late, because a large population of leucocytes was already activated at that time. Thus, timely and well defined periods of leucocyte depletion may offer an advantage over generalized procedures during the whole cardiopulmonary bypass period.

Does leucocyte depletion have effects on other organs? Tang et al. found a better renal function in the leucocyte depleted group in cardiac surgical patients.[80]

Davies et al. used an interesting approach.[81] They removed platelets and leucocytes from patients by plasmapheresis preoperatively. This approach resulted in a reduction of postoperative blood loss, an improved pulmonary function and a

reduction in allogenic blood transfusion. However, their approach has not gained wide acceptance because of the costs and complicated logistics involved. Moreover, it is difficult to accept that a reduction of blood loss could be achieved in the absence of platelets.

From the studies mentioned in this review it may be concluded that leucocyte depletion has a beneficial effect on several clinical parameters. However, leucocyte depletion filters are still relatively little used in routine practice. One explanation may be that there are no large scale studies, which demonstrate the clinical effects of leucocyte depletion in terms of reduced organ injury and length of intensive care unit or hospital stay. There is only one study that comprises 100 patients and in this study it was found that leucocyte filtration applied during all stages of cardiac surgery reduced the inflammatory response after cardiopulmonary bypass, was cost-effective and resulted in a shorter hospital stay.[37] However, this study was designed to compare several anti-inflammatory strategies and was not intended to make an in depth assessment of the effects of leucocyte filtration on perioperative organ injury.

The acceptance of fat processing may have a comparable course. Several studies suggest that the major source of the cerebral microemboli that occur after cardiopulmonary bypass is lipid droplets of the patient's fat that drip into the blood in the surgical field.[20,21] This lipid-laden blood is aspirated and then returned to the patient via the cardiopulmonary bypass circuit. This was the reason for Arrants et al. in 1973 to use a filter for the removal of fat.[23] An ordinary blood filter with a pore size of 35-40 μm was inserted on either the arterial line of the bypass circuit or the cardiotomy suction line or both. Despite a uniform postoperative rise in blood lipids in all studied patient groups, the use of the filter was disappointing as pulmonary and neurological complications did not decrease in the filter group.

Hill et al. used a dacron wool filter in the cardiotomy suction line.[49] They found a reduction in postoperative cerebral dysfunction which was paralleled by a reduction in cerebral microemboli on autopsy studies. Clark et al. studied the fat filtration characteristics of a packed polyester wool filter when used alone in the cardiotomy suction and in the arterial line during clinical extracorporeal circulation.[50] The total lipid extracted from the cardiotomy filters averaged 376 ± 72 mg and from the arterial line filters 512 ± 95mg. They concluded that significant quantities of solids, two thirds of which were fat, were removed by the filters during cardiopulmonary bypass. Most of the fat was derived from the cardiotomy suction system. Recently, Kaza et al. placed an additional filter after the cardiotomy reservoir of the heart-lung machine and looked for circulating fat.[25] There were no large emboli detected after the filter. However, neurocognitive outcome was not measured. In a study in orthopaedic patients after spine fusion, shed wound blood was retransfused either through a 40 μm pore transfusion filter or through a leucocyte removal filter.[82] Leucocytes and fat were measured after the filter. It appeared that a leucocyte removal filter was very effective for the reduction of fat, but that use of a transfusion filter was ineffective.

Realizing that recycling shed blood with cardiotomy suction is an important source of cerebral fat microemboli, Jewell et al. performed a pilot study in patients undergoing cardiac surgery using a cell saver or unprocessed retransfusion of cardiotomy suction blood.[83] They measured the circulating fat. Although they did not find a difference in the postoperative use of blood or blood products, haemoglobin, or bleeding between the two groups, they concluded that use of a cell saver resulted in less fat being recycled during cardiopulmonary bypass. The findings of this study are

in line with a previous study in dogs subjected to cardiopulmonary bypass.[84] An arterial line filter, aimed to reduce leucocytes and fat, was compared with a cell saver, used to process the cardiotomy suction blood. Less cerebral microemboli were observed in the cell saver group. Interestingly, two different types of cell saver were used in this study, which resulted in measurable differences in cerebral microemboli. However, this was a small animal study and the results should be interpreted with caution. In another small clinical study, cardiotomy suction blood and the residual heart lung machine blood were processed by a cell saver.[52] The processed cell saver blood was retransfused using a fat removal filter in conjunction with a leucocyte removal filter. This approach resulted in an improvement of pulmonary function in the filter group vs. unfiltered controls.

These studies suggest that techniques to reduce circulating fat are promising, but further clinical studies are needed to demonstrate effects on outcome.

5. Outline of the thesis

The next four chapters of this thesis consider leucocyte filtration, chapters 6-8 consider the concept and the effects of fat filtration.

Conflicting results have been reported regarding the clinical effects of leucocyte depletion using a leucocyte filter incorporated in the arterial line of the cardiopulmonary bypass circuit. In chapter 2 a new approach is described. We used a leucocyte depletion filter for the residual heart-lung machine blood that was retransfused in the patient after cardiopulmonary bypass. The hypothesis was that blood that is retransfused in a vein first passes the lungs. The lungs act as an endogenous filter and remove activated leucocytes and debris from this blood. If these activated leucocytes and debris were trapped in a filter this would result in less pulmonary injury and improved postoperative lung function.

In chapter 3 we used the same approach in children. The hypothesis was that the effects of leucocyte depletion would be larger than in adults as especially children with a cyanosis would have less possibilities to cope with oxygen radicals that cause postoperative tissue injury.

Chapter 4 addresses the important question whether a leucocyte depletion filter removes all leucocytes, or more specific the activated leucocytes. Depletion of only the activated leucocytes would be beneficial, because these cells play a major role in the reperfusion injury that occurs in the patient after cardiopulmonary bypass as explained in the introduction. In contrast, removal of all leucocytes may be harmful and raise concern about infectious complications.

In chapter 5 the effect of several leucocyte depletion strategies is compared in order to find the optimal approach for leucocyte depletion. The usual application of a leucocyte filter in the arterial line of the cardiopulmonary bypass circuit, the application of a filter in the retransfused heart-lung machine blood, and a novel approach via a venous bypass line in the cardiopulmonary bypass circuit are compared. This last approach was chosen because it would be easy to remove a filter after its use from the circuit during the cardiopulmonary bypass procedure. This is necessary because we feel that leucocytes that are trapped in the filter should subsequently be removed from the circulation to prevent the release of activation products.

Chapter 6 serves as an introduction to chapters 7 and 8 and gives an introduction into the concept of fat filtration. There is evidence that leucocytes, fat and particulate

all contribute to postoperative tissue injury. It may well be that the concept of leucocyte filtration has to be extended to include also fat and particulate.

In chapter 7 the clinical application of a recently developed fat depletion filter is evaluated. This filter was positioned after the cardiotomy reservoir of the heart-lung machine, and thus used for all the cardiotomy suction blood. This blood is known to contain a large amount of fat globules.

In chapter 8 the effects of the fat filtration filter on biochemical markers of brain injury during and after the operation are more closely investigated. Neurocognitive dysfunction is common after cardiac surgery and it is known that cerebral fat microemboli play a role in its origin.

In chapter 9 a summary and conclusions are given.

In chapter 10 a summary in Dutch is given.

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CHAPTER 2

LEUCOCYTE DEPLETION RESULTS IN IMPROVED LUNG FUNCTION AND REDUCED INFLAMMATORY RESPONSE AFTER CARDIAC SURGERY

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ABSTRACT

Leucocyte depletion during cardiopulmonary bypass (CPB) has been demonstrated in animal experiments to improve pulmonary function. Conflicting results have been reported, however, with clinical depletion by arterial line filter of leucocytes at the beginning of CPB. In this study, we examined whether leucocyte depletion from the residual heart-lung machine blood at the end of CPB would improve lung function and reduce the postoperative inflammatory response. Thirty patients undergoing elective heart operations were randomly allocated to a leucocyte-depletion group or a control group. In the leucocyte-depletion group (n = 20), all residual blood (1.2 L to 2.1 L) was filtered by leucocyte-removal filters and reinfused after CPB, whereas in the control group an identical amount of residual blood after CPB was reinfused without filtration (n = 10). Leucocyte depletion removed more than 97% of leucocytes from the retransfused blood ($p < 0.01$) and significantly reduced circulating leucocytes ($p < 0.05$) and granulocytes ($p < 0.05$) compared with the control group. Levels of the inflammatory mediator thromboxane B_2 determined at the end of operation were significantly lower in the depletion group than the control group ($p < 0.05$), whereas no statistical differences in interleukin-6 levels were found between the two groups. After operation, pulmonary gas exchange function (arterial oxygen tension at a fraction of inspired oxygen of 0.4) was significantly higher in the leucocyte-depletion group 1 hour after arrival to the intensive care unit ($p < 0.05$) and after extubation ($p < 0.05$). There were no statistical differences between the two groups with respect to postoperative circulating platelet levels and blood loss, and no infections were observed during the whole period of hospitalization. These results suggest that leucocyte depletion of the residual heart-lung machine blood improves postoperative lung gas exchange function and is safe to be used for those patients who are expected to develop severe inflammatory response after heart operations.

INTRODUCTION

Cardiopulmonary bypass (CPB) induces a whole body inflammatory response that leads to postoperative lung dysfunction.[1-2] This response is largely mediated by the activation of polymorphonuclear leucocytes and by subsequent leucocyte deposition and interaction with the lung endothelium.[3-7] During the initial phase of CPB, leucocytes are activated by the contact of blood with foreign materials in the extracorporeal circuit. After release of the aortic crossclamp in the late phase of CPB, when heart and lungs are reperfused, activation of leucocytes and leucocyte-endothelium interaction are intensified, leading to the impairment of lung function and the induction of a postoperative inflammatory response known as the "post-perfusion syndrome".[8-9]

Leucocyte depletion by means of filtration was originally used by blood banks to prevent transfusion complications associated with donor leucoytes.[10-11] Recent animal experiments demonstrated that leucocyte depletion in different heart operation models reduces heart and lung reperfusion injury.[12-14] Conflicting results have been noted, however, in reports of clinical use at the beginning of CPB of arterial line-filters with leucocyte-depleting capacities.[15-19] Furthermore, there has been concern regarding the simultaneous removal of platelets during leucocyte depletion, which could influence postoperative haemostasis.[13,20]

In this article, we report a study in which only the blood residual in heart-lung machine was depleted of leucocytes, because this blood contains a considerable number of activated leucocytes and is usually reinfused to patients immediately after CPB. We examined whether leucocyte depletion from the residual blood at the end of CPB would improve postoperative lung function and reduce the postoperative inflammatory response. We also examined whether such a "partial" leucocyte depletion method would minimize the major side effect in patients undergoing heart operations, reduction of circulating platelets.

PATIENTS AND METHODS

Patients

After approval by the medical ethical committee in the University Hospital in Groningen and informed consent from patients, 30 patients electively undergoing either coronary artery bypass grafting, heart valve replacement or a combined procedure were randomly allocated to a leucocyte-depletion group (n = 20) or a control group (n = 10). Exclusion criteria were a history of allergy or recurrent infection, reoperation, and emergency operation. The demographic data of patients in both groups are summarized in table 1.

Anaesthesia was induced and maintained by intravenous infusion of sufentanil citrate (1–3 µg/kg) and midazolam (0.05-0.1 mg/kg). Muscle relaxation was achieved with pancuronium bromide (100–140 µg/kg). Cefamandol 2 g and dexamethason 1 mg/kg were administered after induction. Anticoagulation was achieved by intravenous administration of bovine lung heparin at a dose of 300 IU/kg about 5 minutes before the start of bypass.

Table 1. Patient demographic information

	Control (*n* = 10)	Depretion (*n* = 20)
Age (yr)	62 ± 13	60 ± 11
Sex (*n*)		
Male	8	14
Female	2	6
Height (cm)	173 ± 8	172 ± 10
Weight (kg)	80 ± 14	77 ± 11
Body surface (m^2)	197 ± 0.22	19.1 ± 0.16
CABG (*n*)	8	14
AVR (*n*)	-	5
MVR (*n*)	1	-
CABG + AVR (*n*)	1	-
CABG + MVR (*n*)	-	1

Values expressed as mean ± standard deviation of the mean. CABG, coronary artery bypass grafting; AVR, aortic valve replace-ment; MVR, mitral valve replacement.

CPB

The extracorporeal circuit consisted of roller pumps (Stöckert Instrumente, Munich, Germany) and a microporous polypropylene membrane oxygenator (CML Excel, Cobe Laboratories Inc., Lakewood, CO). Within 10 minutes of CPB initiation at a flow rate at 2.4 L/min/m2, the aorta was crossclamped and 1 L of St. Thomas cardioplegia solution (4°C) was infused into the aortic root to provide myocardial preservation. During CPB, moderate hypothermia was induced to maintain the nasopharyngeal temperature between 28 to 30°C. The mean arterial pressure was maintained at 50 to 60 mmHg during CPB. Anticoagulation during CPB was monitored by the celite activated clotting time (International Technidyne Co., Edison, N.J.). After CPB, heparin was neutralised by protamine chloride (3 mg/kg).

Leucocyte depletion

Leucocyte depletion was achieved with the use of RC400 leucocyte-removal filters (Pall Biomedical, Portsmouth, UK) designed particularly for leucocyte filtration under high flow conditions in the operating room.[21] After the termination of CPB, a total volume of 1200 mL to 2100 mL residual blood in the extracorporeal circuit was collected into a blood transfution bag. In the leucocyte-depletion group, the collected blood was filtered by two or three filters and reinfused before the end of operation, whereas in the control group the residual blood was reinfused through the venous transfusion line without leucocyte filtration.

Lung function

Pulmonary gas exchange was measured by the partial arterial oxygen pressure from blood samples drawn from the radial artery line and standardized at a fraction of inspired oxygen of 0.4. Pulmonary haemodynamics exemplified by mean pulmonary artery pressure (PAP) and pulmonary capillary wedge pressure (PCWP) were measured through a Swan-Ganz catheter (Edwards, Baxter Healthcare Corp, Irvine, CA) introduced percutaneously through the right internal jugular vein into the pulmonary artery. Pulmonary vascular resistance (PVR) was calculated according to the following fomula: PVR (dyne.sec.cm^{-5}) = (PAP - PCWP) / CO x 80.

Other clinical parameters

Duration of postoperative intubation was recorded during each patient's stay in the intensive care unit. Blood loss was indicated by 24-hour chest drainage. In addition, durations of stay in the intensive care unit and of hospitalization after operation were obtained from hospital registration records.

Laboratory parameters

For laboratory haematologic tests and biochemical assays, blood samples were taken from the indwelling radial arterial catheter at the baseline before operation, at the end of CPB before transfusion of the leucocyte-depleted blood, at the end of operation during skin closure, 1 hour and 3 hours after the patient's arrival in the intensive care unit, and at 6 am the next day in the intensive care unit. In addition, prefiltration and postfiltration samples were taken from the transfusion bags to determine the cell counts and calculate the rate of leucocyte removal.

Cell counts were determined by a cell counter (Cell-Dyn 610, Sequoia Turner, Mountain View, CA) with a dilution of 1:250 for counting leucocytes and granulocytes and of 1:25,000 for counting platelets. For the postfiltration samples, leucocytes were counted by means of the Nageotte manual counting chamber or by the cell counter with a dilution of 1:100.

For biochemical assays, plasma was obtained by centrifugation of whole blood at 1100 g and stored at -80°C until further determinations. Thromboxane was determined by enzyme immunoassay (Cayman Chemical Company, Ann Arbor, Mich) in plasma anticoagulated with citrate and indomethacin. Interleukin-2 and interleukin-6 were determined by enzyme immunoassay (Quantikine, R&D Systems Europe, Abingdon, UK) from citrated plasma.

Statistics

Data processing as well as statistical tests were performed with the StatView software (Brain-power Inc, Calabasas, CA). Data are expressed as mean plus or minus standard error of the mean unless otherwise indicated. A repeated-measures analysis of variance was used to determine the difference between the two groups. Student's t test or Mann-Whitney test was used for analysis of difference between the two groups at each sampling or recording time point. A p-value less than 0.05 was considered statistically significant.

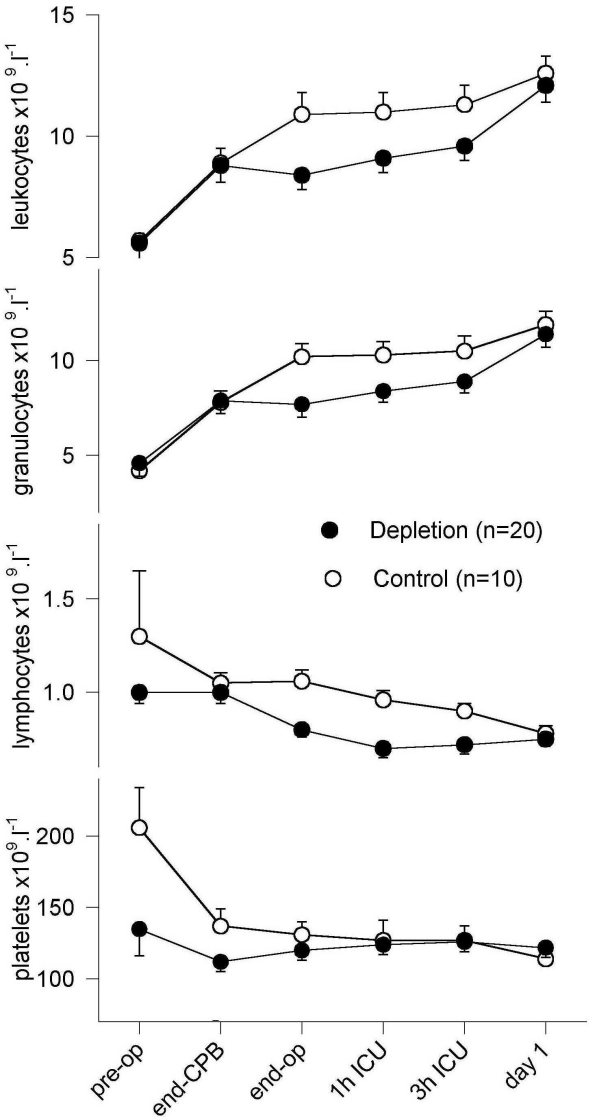

Figure 1. Circulating leucocytes, granulocytes, lymphocytes and platelets in patients receiving and without receiving leucocyte depletion from the reinfused heart-lung machine blood after the end of cardio-pulmonary bypass (CPB). Arrow points the start of depletion (* $p < 0.05$ comparison between the two groups).

RESULTS

There were no significant difference between the leucocyte-depletion group and the control group with respect to duration of CPB and aortic crossclamp time. All patients recovered uneventfully after operation.

Leucocyte reduction in residual machine blood

The average leucocyte count determined from the residual machine blood before filtration was 5.76±0.44 x 10^9/L. After filtration, the count was 0.152±0.01 x 10^9/L. More than 97% of leucocytes were removed from the residual blood in the leucocyte-depletion group. The average platelet count from the machine blood before filtration was 107±6 x 10^9/L, after filtration, it was 43±2 x 10^9/L. About 60% of the platelets in the machine blood were removed by the filters in the leucocyte-depletion group.

Circulating leucocytes and platelets

Circulating leucocyte and granulocyte counts at the end of operation were significantly less in the leucocyte-depletion group than in the control group ($p < 0.05$). There were no significant differences in circulating lymphocyte and platelet counts between the two groups (figure 1).

Inflammatory mediators

Thromboxane B_2 levels were significantly lower in the leucocyte-depletion group than in the control group at the end of operation ($p < 0.05$; table 2). Interleukin-6 levels increased in both the leucocyte-depletion and control groups during the early postoperative period. No significant difference was found between the two groups. Interleukin-2 was not detectable in any of the samples.

Lung function

Pulmonary gas exchange, measured by partial oxygen pressure, was significantly higher in the leucocyte-depletion group than that in the control group both at one hour after arrival in the intensive care unit (118 ± 10 mmHg versus 86 ± 10 mmHg, $p < 0.05$) and immediately after extubation (120 ± 8 mmHg versus 89 ± 10 mmHg, $p < 0.05$, figure 2). PAP was somewhat lower in patients receiving leucocyte depletion than in the control group, but this difference in PAP was not significant. Similarly, there were no statistical differences in PCWP and PVR between the two groups (table 3).

Other clinical outcomes

There was no significant difference between the two groups with respect to postoperative blood loss recorded from the chest drainage until the first postoperative morning. Duration of intubation after operation was slightly shorter in the leucocyte-depletion group than in the control group, but this difference was not statistically significant. Similarly, no statistical difference was found between the two groups regarding the duration of intensive care unit and hospital stay (table 4).

Table 2. Inflammatory mediators before and after operation

Parameter	Before CPB	End CPB	End operation	ICU 1 hr	ICU 3 hr	POD 1
Thromboxane (pg/ml)						
Depletion	ND	48 ± 15	48 ± 9*	33 ± 7	23 ± 29	19 ± 26
Control	ND	62 ± 96	127 ± 63	48 ± 15	59 ± 56	21 ± 34
Interleukin-6 (pg/ml)						
Depletion	36 ± 14	126 ± 96	ND	393 ± 116	344 ± 90	125 ± 46
Control	20 ± 24	197 ± 246	ND	208 ± 103	260 ± 38	155 ± 29
Interleukin-2 (pg/ml)						
Depletion	UD	UD	ND	UD	UD	UD
Control	UD	UD	ND	UD	UD	UD

Values are expressed as the geometric mean and the standard error of the mean. ICU, intensive care unit; POD, postoperative day; ND, not determined; UD, undetectable (below the lowest detectable level stated by the manufacturer).

- $p < 0.05$ compared with control.

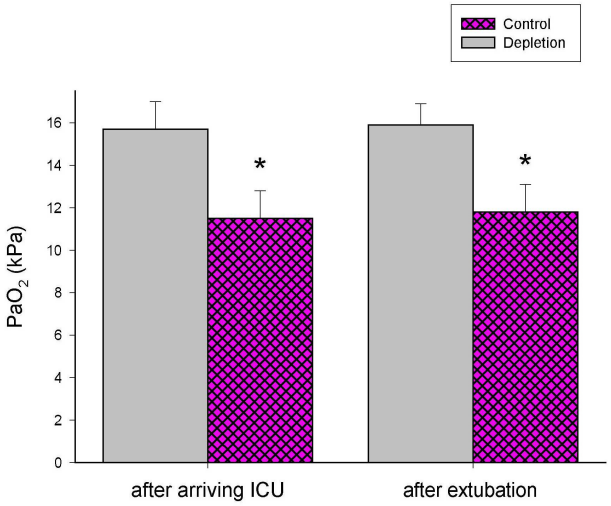

Figure 2. Arterial partial O_2 pressure (PaO_2) determined after arriving in the intensive care unit (ICU) and after extubation in patients receiving leucocyte depletion (Depletion, n = 20) and in patients without receiving depletion (Control, n = 10). The fraction of inspired oxygen was standardized at 40%. (* $p < 0.05$ between the two groups)

Table 3. Pulmonary haemodynamics

Parameter	Before CPB	End CPB	AP	End operation
PAP (mmHg)				
Depletion	16 ± 1.1	17 ± 1.0	14 ± 1.4	17 ± 1.1
Control	17 ± 0.9	20 ± 0.7	19 ± 0.7	21 ± 1.0
PCWP (mmHg)				
Depletion	10 ± 0.9	12 ± 1.4	10 ± 1.5	10 ± 1.2
Control	12. ± 0.7	13 ± 1.1	11 ± 0.9	14 ± 1.3
PVR (dyne.sec^{-1}.cm^{-5})				
Depletion	156 ± 34	129 ± 32	120 ± 23	173 ± 25
Control	145 ± 18	116 ± 22	103 ± 15	125 ± 14

AP, after protamine. Values expressed as mean ± standard error of the mean.

Table 4. Perioperative and postoperative data

	Control (n = 10)	Depletion (n = 20)
CPB time (min)	100 ± 24	94 ± 38
Crosclamp time (min)	67 ± 19	63 ± 29
Blood loss (ml)	685 ± 79	587 ± 87
Intubation time (hr)	13.8 ± 1.3	11.7 ± 0.9
ICU stay (days)	1.1 ± 0.1	1.0 ± 0.0
Hospital stay (days)	8.7 ± 2.1	7.3 ± 0.5

Values expressed as mean ± standard deviation of the mean. CU, intensive care unit.

DISCUSSION

Leucocyte depletion from systemic circulation during cardiopulmonary bypass has been reported to reduce free radical-mediated lung injury and granulocyte-mediated ventricular dysfunction in animal experiments.[12-14] Clinically, however, leucocyte depletion using an arterial line filter at the beginning of CPB has not achieved the goals of reducing intraoperative and postoperative leucocytosis and of improving lung function after heart operations.[15-19] In this study, we have demonstrated that leucocyte depletion of only 1.2 to 2.1 litres residual heart-lung machine blood significantly attenuates postoperative leucocytosis and improves pulmonary gas exchange function in patients undergoing heart operations. Of more importance, because this blood is usually transfused through the venous line without any substantial filtration, leucocyte depletion in this setting may have provided a local protective effect for the lungs, even if the amount is relatively small.

There are at least two reasons why leucocyte depletion of the residual heart-lung machine blood may protect the lung. First, we observed in a recent study[22] that the residual heart-lung machine blood contained higher levels of leucocyte release products than seen in the systemic circulation, suggesting that the leucocytes remaining in the heart-lung machine are highly activated. Second, it is known that the heart-lung machine blood contains a number of foreign substances as well as microaggregates formed mainly by platelets and leucocytes.[23,24] During CPB, the blood is returned to patients from the arterial side of the heart-lung machine, where an arterial line filter removes microaggregates. After CPB, however, the residual heart-lung machine blood is reinfused to patients via intravenous transfusion without any substantial filtration (usually only a clot filter with large pore size is used). Because the lung is anatomically located to receive all the reinfused blood from venous side, lung injury may occur as a result of the pulmonary accumulation of micro-aggregates[25,26] mediated by trapped platelets and leucocytes.

In fact, current leucocyte-depleting filters remove not only leucocytes but also other particulates less than 5 μm in diameter. It has been reported recently that a similar type of blood transfusion filter was able to remove the microfibrillar collagen haemostat from the wound blood harvested from the surgical field.[27] Particulate microaggregates are continuously generated during CPB; this is particularly evident in the cardiotomy returning line.[23] These microaggregates are mostly smaller than 30 μm in diameter[23] and are not always caught by the cardiotomy filter which usually has a pore size between 20 to 40 μm.[28] Because the residual machine blood collected at the end of CPB contains a large portion of blood from the cardiotomy reservoir, filtration with a leucocyte-removal filter may prevent any particulates larger than 5 μm from being retransfused to patient, thereby reducing lung injury.

Although a direct comparison of our results with results obtained from arterial line leucocyte depletion is unjustified, it does appear that leucocyte depletion of the residual machine blood is more likely to have a local effect on protecting the lungs. In addition, leucocyte depletion with transfusion filters may have other advantages in clinical application. The procedure is easy to handle because the filter can be installed at any time before use without flush or priming. Moreover, it could serve as an optional intervention method that can be added at the end of CPB according to patient's clinical condition, particularly for patients with a longer duration of CPB and a predicted strong postoperative inflammatory response. One potential disadvantage

of this method, however, is the limited blood volume available for filtration, which depends on the volume of residual blood in the heart-lung machine.

The inflammatory mediator thromboxane B_2 is usually increased during and after CPB in patients undergoing heart operations.[29] In this study, we observed a significantly reduction of plasma thromboxane B_2 at the end of operation in the leucocyte depletion group compared with the control group; this difference can be explained by the removal of activated leucocytes and the simultaneous removal of platelets after the end of CPB. We also measured interleukin-6 and interleukin-2; the former is a marker of acute-phase response produced by mononuclear phagocytes and the latter is mainly produced by lymphocytes.[30] We confirmed that the peak release of interleukin-6 occurred about 1 hour after arrival in the intensive care unit, as reported by other groups.[31] No significant difference was found between the depletion and the control groups, however, which suggest that leucocyte depletion in this setting has no effect on the release of interleukin-6 during the early postoperative period. Interleukin-2 was not detectable in any samples, indicating that there was no lymphocyte-associated release of cytokines in these patients. This is in agreement with a recent report that interleukin-2 could be detected only occasionally after heart operations.[32]

One of the concerns regarding leucocyte depletion during heart operations is that the simultaneous removal of platelets[13,17] might affect postoperative haemostasis. In this study, little influence on circulating platelet count was observed in patients receiving leucocyte depletion, although considerable numbers of platelets were removed from the reinfused heart-lung machine blood. Consistently, there was no significant difference between the two groups with respect to the postoperative blood loss. On the other hand, it remains to be elucidated whether removal of platelets from the residual heart-lung machine blood contributed to improved postoperative lung function. It is known that the platelets may deposit in the myocardium during reperfusion,[33,34] leading to myocardial reperfusion injury.[35] Moreover, release products from platelets such as platelet activating factor and platelet associated adhesive molecules may further activate leucocytes and promote leucocyte adhesion to the endothelium.[36-38] This mechanism may also operate in initiating lung injury because platelet deposition occurred similarly during lung reperfusion in the lung microvasculature.[39]

In conclusion, leucocyte depletion from residual heart-lung machine blood at the end of CPB improves postoperative lung gas exchange function and reduces postoperative leucocytosis. Furthermore, leucocyte depletion in this setting did not result in any postoperative complications with respect to haemostasis and infection. Further investigations should be carried out to compare the different leucocyte depletion methods with respect to their clinical benefits against costs, and to determine which patient populations can profit most from this intervention.

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CHAPTER 3

LEUCOCYTE FILTRATION OF RESIDUAL HEART LUNG MACHINE BLOOD IN CHILDREN UNDERGOING CONGENITAL HEART SURGERY

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ABSTRACT

Cardiopulmonary bypass (CPB) leads to a generalized inflammatory reaction, resulting in increased postoperative leucocyte counts and decreased pulmonary function. In adults, removal of leucocytes from the residual heart-lung machine blood after CPB improved postoperative oxygenation. In children, however, the clinical effects of leucocyte filtration of the residual heart-lung machine blood are unknown. Therefore, we measured postoperative leucocyte counts and arterial blood oxygenation in children undergoing congenital cardiac surgery in a randomized prospective study. Anaesthesia and CPB were standardized. After CPB, the residual heart-lung machine blood was collected as usual. In a group of 25 children, this blood was filtered with a leucocyte depletion filter before transfusion. A control group of 25 children received this blood unfiltered. We found that the postoperative leucocyte counts were significantly lower in the filter group than in the control group (p = 0.02, repeated measurements ANOVA). This difference reached a maximum on the second postoperative day (12.9 x 10^9/L filter vs. 15.9x10^9/L control, p = 0.02, Student's t-test). Values for the arterial blood oxygenation on the first postoperative day were not different between both groups (15.5 ± 1 kPa filter vs.14.6 ± 1.3 kPa control, p = 0.57, Student's t-test). We conclude that leucocyte filtration of the residual heart lung machine blood reduced systemic leucocyte counts, but did not improve arterial blood oxygenation in children after congenital heart surgery.

INTRODUCTION

Cardiopulmonary bypass (CPB) leads to a systemic inflammatory reaction mainly through activation of complement and leucocytes, resulting in increased postoperative leucocyte counts and a decreased pulmonary function.[1-3] In children, the inflammatory response to CPB tends to be more intense than in adults, because the surface of the CPB circuit in relation to the total body volume is larger.[4] Moreover, this leucocyte-mediated inflammatory response initiated during CPB is likely to have a significant clinical inpact, as a negative correlation between the expression of leucocyte adhesion molecules and postoperative oxygenation has been demonstrated recently in children.[5]

Leucocyte filtration is a recently introduced technique to reduce a CPB-induced inflammatory response.[6,7] Previously, we have shown in adults that leucofiltration of the residual heart-lung machine blood after CPB resulted in a reduced inflammatory response and improved lung function.[8] In this study we examined the effect of leucofiltration of residual heart-lung machine blood on postoperative oxygenation and circulating leucocyte counts in children undergoing congenital heart surgery.

METHODS

After ethical committee approval and parent consent, 50 consecutive children who underwent congenital open-heart surgery were randomly divided into two groups. Procedures selected were correction for tetralogy of Fallot, simple closure of a ventricular septal defect, correction of atrioventricular septal defect, arterial switch operation for transposition of the great arteries and completion of the Fontan procedure. The power calculation for this study was based on the results of the partial oxygen pressure of the arterial blood (PaO_2) in our study in adults.[8] The PaO_2 on the first postoperative day was the primary end point for this study. It was therefore estimated that with an α 0.05 and a β of 0.8, a total of 45 patients would be required to reach a statistically significant difference. Thus, in 25 children, the residual blood from the heart-lung machine after CPB was filtered with a leucocyte depletion filter before retransfusion. In a control group of 25 children this residual blood was retransfused unfiltered.

Anaesthesia was standardized and consisted of a midazolam and sufentanil infusion. Pancuroniumbromide was used for muscle relaxation. Ventilation was aimed at normocapnia with oxygen in air (FiO_2 = 0.4), a tidal volume of 6-10 $ml.kg^{-1}$ and a positive end-expiratory pressure of 2-4 cmH_2O. CPB was instituted after heparin (300 IU/kg^{-1}) was given. The bypass circuit consisted of a double head roller pump (Stöckert, München, Germany) and a hollow fibre oxygenator (Dideco safe micro or Dideco 902, Sorin, Mirandola, Italy, depending on the size of the child). The flow during CPB was 2.4 L/m^2 with moderate hypothermia (± 30°C). The priming solution consisted of human albumin 5% with 1000 IU heparin. If the calculated haemoglobin on bypass was less than 4.5 mmol/L, packed cells were added to the priming solution. After CPB and disconnection of the system, the residual blood in the heart-lung machine was collected in a transfusion bag, and retransfused in the child during wound closure and the first 2 hours in the intensive care unit (ICU). In the filter group this blood was filtered with one leucocyte depletion filter (Pall RS 1, Pall, Portsmouth,

GB) for each patient. Other filtration procedures (e.g. modified ultrafiltration) were not used.

We made the following measurements in the children. Leucocyte counts were performed after induction of anaesthesia and on the first four postoperative days. Platelet counts and the PaO_2 were determined after induction of anaesthesia, after arrival in the ICU and on the first postoperative day. In 10 children additional blood samples were taken from the residual heart-lung machine blood. In these samples leucocyte and platelet counts, and levels of haemoglobin and elastase, as a measure of leucocyte activation, were determined.

The statistical analysis was done as follows. For comparison of single data between the groups a two tailed Student's *t*-test or the non-parametric Mann-Whitney test was used as appropiate. For the comparison of the groups for oxygenation, platelet and leucocyte counts on the different time points two way analysis of variance (ANOVA) for repeated measures was used to identify time, group and time-group interactions. To allow for multiple comparisons the Bonferroni adjustment was applied. Values are given as mean and standard error of the mean for normal distributed data, otherwise the median value and the 25 and 75 percentiles are given. A p-value ≤ 0.05 was considered significant.

RESULTS

The demographic data were similar in both groups (table 1). Intraoperatively, 270 ± 27 mL of residual heart-lung machine blood was collected in a transfusion bag after CPB. This corresponded with an index of 707 ± 50 mL/m^2. The composition of the residual heart-lung machine blood is shown in table 2.

The postoperative leucocyte counts increased over time ($p < 0.001$, figure 1), with a significant difference between the filter and the control group ($p = 0.02$). Post-operative platelet counts were not different between the groups ($p = 0.11$, repeated measurements ANOVA, table 3).

The PaO_2 values were similar in both groups ($p = 0.43$, repeated measurements ANOVA, table 3). However, to investigate if the underlying disease of the children influenced the postoperative PaO_2 values, we divided the children in a subgroup with a preoperative $PaO_2 = <8.4$ kPa and in a subgroup with a $PaO_2 > 8.4$ kPa. This arbitrary value parallelled the division in underlying cyanotic and non-cyanotic disease. The results, shown in table 4, indicate that in the cyanotic group (n = 28) the children in the filter and the control group had a similar postoperative PaO_2, whereas in the non-cyanotic group (n = 22) the children in the filter group had a slightly higher post-operative PaO_2.

The haemodynamic data at the end of the operation were not different (table 3). However to achieve that result dopamin (5-10 μg/kg/min) was used in 21 children in the control group vs. 11 in the filter group ($p = 0.004$) and isoprenalin (0.01-0.02 μg/kg/min) was used in 11 children in the control group vs. 2 in the filter group ($p = 0.004$). Blood loss during the first 24 hours was not different (table 3).

Table 1. Demographic data

	Filtration ($n = 25$)	Control ($n = 25$)	p-value
Surgical procedure			
Closure of AV-canal	6	5	
Correction of Fallot	6	7	
Completion Fontan	4	5	
Arterial switch	6	5	
VSD	3	3	
Age (month)	13 (2 – 31.5)	6 (1 - 28.5)	0.44
BSA (m^2)	0.42 (0.25 – 0.58)	0.35 (0.25 - 0.54)	0.50
CPB (min)	119 ± 8.7	126 ± 8.3	0.58
Intubation time (hour)	19 (9 - 48)	21 (16 - 54)	0.36
ICU stay (day)	1 (1 - 3.7)	2 (1 - 5)	0.27
Hospital stay (day)	8 (7 - 11.7)	8 (7.5 - 13)	0.43

AV, atrioventricular; VSD, ventricular septal defect; BSA, body surface area; CPB, cardiopulmonary bypass; ICU, intensive care unit

Table 2. Composition of the residual heart lung machine blood in 10 children

Leucocytes ($x10^9$./L)	5.1 ± 0.53
Haemoglobin (mmol/L)	4.5 ± 0.28
Platelets ($x10^9$/L)	141 ± 16
Elastase ($ng.L^{-3}$)	70 ± 54

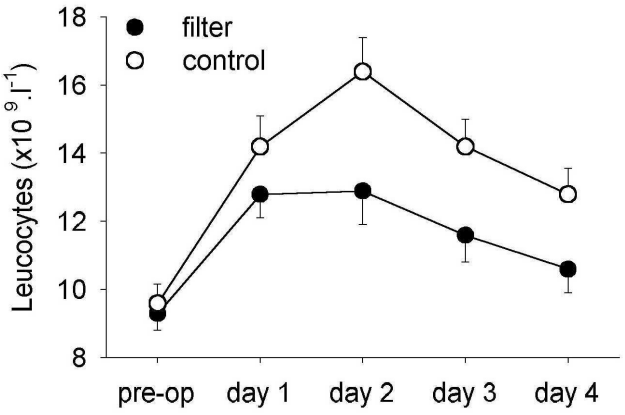

Figure 1. Mean leucocyte counts before (pre-op) and after surgery for congenital heart disease (postoperative day 1, 2, 3 and 4) in children, that had the residual heart-lung machine blood filtered with a leucocyte depletion filter before retransfusion (filter), and in children, that had this blood retransfused unfiltered (control). The error bars represent the standard error of the mean. Repeated measurements analysis of variance revealed a significant difference over the time ($p < 0.001$) and between the two groups ($p = 0.02$)

Table 3. Clinical data

		Filtration ($n=25$)	Control ($n=25$)	p-value
Oxygenation (kPa)	Pre-op	11.4 ± 1.3	10.8 ± 1.3	0.77
	Arrival ICU	16.7 ± 1.3	14.8 ± 1.3	0.36
	Day 1 ICU	15.5 ± 1	14.6 ± 1.3	0.57
Circulating platelet count ($x10^9.l^{-1}$)				
	Pre-op	327 ± 18	380 ± 38	0.20
	Arrival ICU	126 ± 10	154 ± 11	0.07
	Day 1 ICU	162 ± 13	181 ± 13	0.31
Heart rate	Arrival ICU	140 ± 4	142 ± 3	0.79
MAP (mmHg)	Arrival ICU	55 ± 1.7	59 ± 2	0.17
CVP (mmHg)	Arrival ICU	11.3 ± 0.5	12.8 ± 0.6	0.08
Blood loss ($ml.m^{-2}$)		414 ± 68	324 ± 34	0.27

Pre-op, pre-operative; ICU, intensive care unit; Day 1, the first postoperative day; AP, mean arterial pressure; CVP, central venous pressure

Table 4. Postoperative partial oxygen pressure of the arterial blood in cyanotic and non-cyanotic children receiving leucocyte filtration

Preoperative PaO_2	< 8.4 kPa			> 8.4 kPa		
Group	filter ($n=14$)	control ($n=14$)	p-value	filter ($n=11$)	control ($n=11$)	p-value
Pre-op	6.7 ± 0.3	6.8 ± 0.4	0.9	17.2 ± 1.5	16.0 ± 2.0	0.63
Arrival ICU	15.6 ± 1.8	16.0 ± 2.1	0.88	17.7 ± 1.6	13.2 ± 1.6	0.06
Day 1 ICU	14.4 ± 1.2	15.1 ± 1.9	0.76	17.0 ± 1.5	14.0 ± 1.7	0.21

PaO_2, partial oxygen pressure of the arterial blood; pre-op, preoperative; ICU, intensive care unit; Day 1, the first postoperative day

DISCUSSION

Although the leucocyte depletion technology during cardiac surgery has repeatedly been reported in adults, only limited information is available in children.[9-12] This study shows that in children undergoing cardiac surgery leucofiltration of residual heart-lung machine blood resulted in a prolonged reduction of postoperative leucocyte counts. However, in contrast to adult patients, this did not result in a difference in postoperative PaO_2.

There are at least two possible explanations for the difference between adults and children regarding the clinical effects. In the first place, we found high elastase values in the residual blood, which were about twice the values that we previously found in the residual heart-lung machine blood in adult patients.[13] The elastase values reflect

the degranulation of leucocytes and are associated with tissue injury. Thus, although the number of leucocytes in the residual blood is somewhat lower than in adults,[13] it is likely that, after CPB, children have more activated leucocytes. Moreover, an increase in leucocyte adhesion molecules has been demonstrated in children from the first to the second postoperative day together with an increase in neutrophil counts, opposed to a decrease in adhesion molecules and neutrophil counts in adults.[14] This could explain why we found a more prolonged effect of leucofiltration on leucocyte counts in children than in adults, where the leucocyte counts on the first postoperative day were similar in the filter group and in the control group.[8] Second, the moment, or timing, of leucocyte filtration during the operation may also play an important role in its clinical significance and this could explain why we did not find an improvement in PaO_2. Children, especially children with cyanotic heart diseases have a low tolerance for free oxygen radicals, and it is therefore likely that the damaging effects of activated leucocytes occur in the initial reperfusion phase, immediately after aortic cross-clamp release.[9] This has also been shown as an increase in myeloperoxidase and lactoferrin, as markers for leucocyte activation, immediately after aortic cross clamp release.[15] Thus in the cildren in the cyanotic group, with the large difference in pre- and postoperative PaO_2 values, leucofiltration of the residual heart-lung machine blood was too late to have an effect, which may explain why the postoperative PaO_2 values in the filter and the control group were similar. In contrast, the children in the non-cyanotic group, with no difference in pre- and postoperative PaO_2 values, had no oxygen stress and thus in this small subgroup statistical significance in the postoperative PaO_2 values between the filter and the control group was nearly obtained. Currently, leucocyte depletion filters suitable for use in a paediatric extracorporeal circuit are not available, which prevents earlier use of leucofiltration during the operation. A paediatric arterial-line filter has been used, but was withdrawn due to reported clotting in the CPB circuit distal to the filter[10]. Thus, our strategy for children may be too late for an effect on oxygenation, but sufficient for an effect on leucocyte counts.

Although platelets are removed by leucocyte depletion filters,[16] there was no significant difference between the two groups regarding the postoperative platelet counts. This finding may also explain that the postoperative blood loss was not different between the two groups. The amount of retransfused residual blood in the children, indexed on body surface, was comparable to that in the adult patients.[8] We transfused this blood during wound closure and the first 2 hours in the ICU. This was not due to a flow limitation in the filter, but to the capacity of the child to accept the transfused volume. For each patient we used one filter, which costs about 30 euro. However, a larger study is necessary to assess if the use of these filters is cost-effective.

An unexpected finding was that more inotropes were used in the control group. This was transient, because on the first postoperative day this difference was not present. Reduced inotropic support after leucofiltration has been described previously.[12,17] Our results support these findings and suggest that even leucofiltration performed shortly after CPB results in a more stable haemodynamic profile.

Our study may be limited by the fact that we did not measure biochemical parameters such as elastase or interleukins in the children. The measurement of biochemical parameters would, of course, be helpful to gain insight in the effects of

leucocyte depletion, but, for the widespread application of a filtration strategy, it is necessary to obtain clinically useful results, which was on our primary clinical endpoint, an improved postoperative PaO_2 in the filter group, not the case in this study. A second limitation is the heterogenous patient group. We tried to minimise this by the selection of well described disease entities, and by a post hoc analysis of the effects of leucofiltration in cyanotic and non-cyanotic children. However, the pre- and postoperative PaO_2 should be interpreted with caution.

In conclusion, we found a significant reduction in leucocyte counts in children after corrective surgery for congenital heart disease by the transfusion of leucocyte depleted residual heart-lung machine blood. However, we did not find significant effects on postoperative oxygenation. Since the elastase levels in the residual heart-lung machine blood are high, and there is currently no suitable paediatric arterial in-line filter to achieve leucocyte depletion during CPB, the removal of leucocytes from this blood may offer an alternative to reduce the inflammatory reaction in children after congenital heart surgery.

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CHAPTER 4

FILTRATION OF ACTIVATED GRANULOCYTES DURING CARDIOPULMONARY BYPASS SURGERY: A MORPHOLOGIC AND IMMUNOLOGIC STUDY TO CHARACTERIZE THE TRAPPED LEUCOCYTES.

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ABSTRACT

Cardiopulmonary bypass surgery induces an inflammatory reaction among others by granulocytes. Leucocyte filtration has been shown to reduce the postoperative morbidity mediated by activated granulocytes. However, little is known about the mechanism of filter-leucocyte interaction. This study examines whether a leucocyte filter removes activated granulocytes or a general leucocyte population.

Eleven patients undergoing cardiopulmonary bypass surgery were included in this study. Leucocyte filtration was achieved before the reperfusion phase with a Pall non-woven polyester filter located at the venous side of the heart-lung machine. After filtration, the trapped granulocytes inside the filter were examined with light and scanning electron microscopy and immunologically by CD45RO antigen binding to the filter material. Furthermore, leucocyte release markers were measured to determine whether cells were activated during filtration.

On microscopic evaluation it was found that 84% granulocytes and 14% lymphocytes were trapped in the filter, compared with 78% granulocytes and 22% lymphocytes in the blood before filtration. Granulocytes were trapped significantly more in the first blood contact layer of the filter material than in the middle layer and last layer, whereas lymphocytes trapped slightly more in the middle layer. The near maximum level of CD45RO expression was measured on granulocytes trapped inside the filter material, whereas CD2 and CD19 measured on lymphocytes were bound to a minor extend. ß-Glucuronidase concentration did not increase after filtration, suggesting the absence of activation of granulocytes by filtration.

The results of this study suggest that a leucocyte filter made of non-woven polyester material removes the activated granulocytes rather than leucocytes at random. This implies that this particular type of leucocyte removal filter is suitable for use in cardiopulmonary bypass patients whose granulocytes in the circulation are activated. Furthermore, measurement of activated granulocytes instead of total leucocyte count is likely preferable for functional assessment of leucocyte removal devices.

INTRODUCTION

Foreign materials used in the Cardiopulmonary bypass (CPB) circuit during heart surgery activate leucocytes, resulting in increased cell adhesiveness, release of oxygen radicals and enzymes, and finally, damage to the host.[1-9] Primarily the neutrophilic granulocyte fraction is activated after initial contact with extracorporeal surfaces.[3-7] It has been suggested that removal of these activated granulocytes by filtration reduces morbidity after heart surgery.[10-15] However, during leucocyte filtration in CPB procedures, we observed a large patient-related variation in filtration efficiency.[16] Based on the suggested mechanism of leucocyte removal by synthetic filters – that is, by adhesion to the filter material rather than by seaving[17] – we speculated that this variation in filtration efficiency was related to a difference in the expression of leucocyte receptors, leading to a difference in adhesion capacity to the filter material. However, it has never been reported, by directly studying the leucocyte-filter interaction, whether during CPB, leucocyte filters remove granulocytes at random or remove primarily an activated subset of granulocytes. Therefore, we designed this study to examine whether leucocyte filters remove a large portion of activated granulocytes or a general leucocyte population. To achieve this goal, we used a filter during 14 minutes in the clinical setting of CPB and performed electronic cell count and biochemical tests on blood samples taken before and after filtration. Additionally, we performed histologic examination on embedded filter material and immunologic tests to show the presence of 'activation receptors' on leucocytes trapped inside the filter and on whole blood samples before and after filtration.

METHODS

Patients

After approval was received from the ethical committee and informed consent was received from patients, 11 patients undergoing an elective heart operation for either coronary artery bypass grafting or heart valve replacement were included in the study. Exclusion criteria were a history of allergy or recurrent infection, reoperation, and emergency operation. The characteristics of the patients are summarized in table 1.

Heart operation procedure

Anaesthesia was induced and maintained by intravenous infusion of sufentanil citrate (1 to 3 μg/kg) and midazolam (0.05 to 0.1 mg/kg). Muscle relaxation was achieved with pancuronium bromide (0.1 mg/kg). Cefamandol at a dose of 2 g and dexamethasone at a dose of 1 mg/kg were administered after induction. Anticoagulation was achieved by intravenous administration of bovine lung heparin at a dose of 300 IU/kg approximately 5 minutes before the start of CPB. Anticoagulation was monitored by Celite activated clotting time (International Technidyne Co, Edison, NJ). After CPB, heparin was neutralized by protamine chloride (3 mg/kg). The heart-lung machine consisted of roller pumps (Stöckert Instrumente GMBH, Munich, Germany) and a microporous polypropylene membrane oxygenator (CML Excel; Cobe Laboratories Inc., Lakewood, CO). Within 10 minutes of CPB initiation at a flow rate at 2.4 L/min/m^2, the

Table 1. Characteristics of patients (n = 11) receiving filtration.

Variable	unit	mean (range)
Age	Y	63 (47 - 76)
Male		10
Female		1
Height	Cm	175.1 (165 - 195)
Weight	Kg	79.6 (60 - 103)
CPB time	min	87.7 (56 - 165)
X-clamp time	min	60.5 (38 - 103)
Lowest temperature (np) during CPB	^{0}C	30.2 (27.6 - 31.7)
CABG		9
AVR		2

CPB, cardiopulmonary bypass; X-clamp, aortic cross-clamp; CABG, coronary artery bypass grafting; AVR, aortic valve replacement

aorta was cross-clamped and 1 L St. Thomas cardioplegic solution (4^{o}C) was infused into the aortic root to provide myocardial preservation. During CPB, moderate hypothermia was induced (table 1). The mean arterial pressure was maintained at 50 to 60 mmHg during CPB.

Leucocyte filtration

Leucocyte filtration was achieved by using a prototype leucocyte removal filter (B 1320A; Pall Biomedical, Portsmouth, England). This was a redesign of the prototype filter used for our previous study[16] to make it easier for clinical handling. The filter was incorporated in the circulation, in a parallel circuit at the venous site of the heart-lung machine, and one of the roller pumps (Stöckert) was used to maintain a flow rate of 500 mL/min. Leucocyte filtration was performed during the rewarming phase at the end of CPB just before release of the aortic cross-clamp and lasted for approximately 14 minutes. During filtration the pressure at the inlet side of the filter averaged 74 ± 17.5 mmHg.

Blood sampling

Blood samples were taken before and after filtration from the radial artery of the patients and every 2 minutes during filtration from the inlet and outlet sides of the filter. The blood specimens were collected in sodium citrate (0.32%). Leucocyte counts were performed with an electronic cell counter (Cell-Dyn 610; Abbott, Santa Clara, CA) to asses leucocyte removal by the filter. The relative cell removal rate was calculated every 2 minutes according to the following formula: relative cell removal rate = (1-[post-filter count/pre-filter count]) x 100. The average cell removal was calculated as a mean of the relative removal rates. The total number of removed cells was calculated by multiplying of the absolute number of removed cells per liter (post filter count minus pre-filter count) with the volume of filtered blood. For biochemical assays, plasma was obtained by centrifuging of whole blood at 4°C for 10 minutes at 1100g, whereafter plasma was stored at -80°C until further examination. ß-glucuronidase, a release product of activated granulocytes, was determined by an enzymatic assay (photospectrometry; Boehringer,

Mannhein, Germany) in plasma samples from the inlet and outlet sides of the filter, after 8 minutes of filtration, to indicate activation of granulocytes by the filter material. Platelet activating factors inhibiting capacity (PAF-IC) was also determined from the inlet and outlet sides of the filter after 2 minutes filtration by turbidometry in an aggregometer (Chrono-Log, Havertown, PA) to indicate platelet activating factor (PAF) production by activated leucocytes. The measurement of PAF-IC was conducted with platelets isolated from citrated platelet-rich plasma containing indomethacin (50 µg/mL) from the blood of a healthy volunteer by filtration through Sepharose CL-2B (Pharmacia Biotech Inc., Stockholm, Sweden). These platelets were resuspended in saline to a final platelet concentration of 50 x 10^9/L and added to the plasma of the study patients. The maximum velocity of platelet aggregation was measured after PAF C16 (Cayman Chemical, Ann Arbor, MI) addition and was used to indicate the PAF-IC of the patients' samples. Because the PAF-IC is maximal in normal plasma and reduces after PAF formation, normal human plasma was used as a negative control and saline as a positive control, resulting in no aggregation of the platelets and maximum aggregation of the platelets, respectively.

Morphologic examination of leucocyte entrapment

Nine leucocyte filters were collected immediately after CPB and were prepared for histological examination to enable differential leucocyte counting in the cross-section of the filter material. After release of the residual blood, the filters were perfused in their indicated flow direction with 500 mL of normal saline solution under a consstant pressure of 75 mmHg to wash away the unbound leucocytes. This low perfusion pressure did not exceed the clinical filtration pressure and was chosen to prevent the release of attached leucocytes by high shear forces. To further control the stability of leucocyte binding within the filter, part of the filter material was washed again after the standard procedure with 3 L of normal saline solution under similar perfusion pressure. In each filter, leucocytes were counted in 3 different layers of the cross-section. This comparison showed that washing the filter with 500 mL of normal saline solution did not differ significantly with results when washing with 3 L of saline solution in regard to bound leucocytes (table 2) and thus was used as a standard procedure for the future experiments. After washing, the filters were cut open without damaging the filter material, and within 60 minutes after filtration, partly fixated in 4% paraformaldehyde 0.1 mol/L phosphate buffer (pH 7.4) and stored at -20°C. In duplicate, samples of the fixed filter material were dehydrated with alcohol and distilled water and embedded in plastic (GMA, Technovit 8100). Series of three slices of 2 µm were cut out of the cross-section of both samples of the plasticized filter material with a microtome (Jung 1140) and a D-knife with a Tungsten Carbide edge (16/20). Thus in total, 6 samples of each filter were prepared for light microscopy. All slices were stained by standard histologic methods with May-Grunwald-Giemsa and viewed under the microscope. Leucocytes in each slice were counted on three locations of the cross-section of the filter material. As the first location, the first layer of the filter material touched by blood was chosen. As the second location, the middle layer of the cross-section was viewed. As the third location, a microscopic view of the last layer, which bordered on the outlet of blood from the filter, was examined. Differential counting for segmented neutrophilic granulocytes,

Table 2. Mean differential microscopic leucocyte count in three layers between two groups

	*Normal Washed**			*Extra Washed**		
	1	*2*	*3*	*1*	*2*	*3*
Segmented neutrophils	278.6	86.6	47.1	282.3	70.6	29.7
band neutrophils	16.4	7.7	3.7	17.4	4.7	1.3
basophilic granulocytes	0.8	0.3	0.1	0.6	0.2	0.0
eosinophilic granulocytes	17.6	2.0	0.1	18.8	1.9	0.3
lymphocytes	25.2	28.2	19.7	24.4	30.7	21.3
monocytes	9.6	2.5	1.6	11.6	2.1	0.7

Samples were washed according to protocol with 500 mL saline solution (n = 24, 3 different filters), and samples were extra washed with 3 L saline solution (n = 18, 3 different filters). No statistically significant differences were observed between these two washing procedures.
*layers: 1, first blood contact layer; 2, middle layer; 3, last blood contact layer.

band neutrophilic granulocytes, lymphocytes, monocytes, eosinophilic and basophilic granulocytes was done in one microscopical field (enlargement x400) under the condition that at least 100 cells were counted in one layer. For scanning electron microscopy, pieces of filter material out of the same three layers that had been fixed were used. Postfixation was performed with 1% OsO_4 in phosphate-buffered saline solution for 3 hours, followed by dehydration in ethanol series. After critical point drying with CO_2, the samples were supercoated with gold and examined with scanning electron microscopy at 2 kV (Jeol 6301F, Tokyo, Japan).

Immunologic examination of activated granulocyte entrapment

The freezer-stored non-fixed parts of the filters were examined for the binding of specific antibodies to cells trapped inside the filter material. The antibodies used for this procedure were labeled with europium Eu-DDTA (Wallac, Turku, Finland), which allows sensitive detection by means of time-resolved fluorescence.[18] Mouse anti-human CD45RO monoclonal antibody (Caltag Laboratories, San Francisco, CA) was used as a marker for the specific binding of activated granulocytes. To estimate the amount of CD45RO binding to T and B cells, specific antibodies against T-cell receptors (mouse anti-human CD2 monoclonal antibody; Caltag Laboratories) and against B-cell receptors (mouse anti-human CD19 monoclonal antibody; Caltag Laboratories) were used. To measure the binding of the antibody to the cells trapped inside the filter material, materials were separated in three layers representing the same specific locations of the cross-section of the filter material used in the morphologic examination. Each layer was divided into three parts. This resulted in nine parts per filter to be tested. Each part was carefully weighed to correct the amount of antibody binding for filter mass. Then, through a standard procedure, each part was washed with saline solution and incubated for 30 minutes with Eu-labeled antibody on a plateshaker and for 5 minutes with 3% H_2O_2. After the non-bound antibody was washed away, the Eu was released in enhancement fluid and counted in an Arcus (Wallac). A negative reference was made during each test by triplicate measurement of the nonspecific antibody binding to a sample of non-used filter material that had been incubated for 60 minutes in leucocyte-

and platelet-free plasma. A positive reference was made on filter material samples from 3 patients to test the maximum CD45RO binding to the filter material; Zymosan-activated plasma (Sigma, St. Louis, MO) containing high concentrations of C5a was incubated with the filter material for 20 minutes before CD45RO antibody binding.[19,20]

In addition, removal of activated leucocytes from the blood of patients during CPB was tested by flow cytometric measurement of the adhesive receptor present on activated granulocytes (CD11b; DPC, Los Angeles, CA) in blood before and after the filter. Thus from 3 patients, blood samples were taken before filtration from the radial artery, before and after passing the filter at 2 and 10 minutes filtration from the afferent and efferent lines of the filter, and after the filtration procedure from the radial artery. Immediately after collection in sodium citrate (0.32%) blood was incubated with phyco-erythrin-labeled anti-CD11b, treated with Optilyse C (Immunotech, Marseilles, France), and prepared for flowcytometric analysis (FACS, Coulter, Luton, England).

Statistics

Before data analysis, all non-categorical data were tested and found normal distributed according to the Kolmogorov-Smirnov goodness-of-fit test. An unpaired two-tailed Student *t* test was used to test the differences between the different leucocyte counts, non-categorical patient characteristics and immunologic data. An unpaired one tailed Student *t* test was used to test the difference between the rinsed and extra-rinsed microscopical leucocyte counts. To detect possible differences between microscopic cell counts in the three different layers in cross-section, one way analysis of variance was used to compare groups. Duncan's multiple comparison post hoc procedure was used to quantify any differences among groups that were found to be significant. A value of $p < 0.05$ was considered statistically significant. All haematologic, morphologic, immunologic and biochemical data are expressed as mean and standard error of the mean, unless otherwise indicated.

RESULTS

Morphologic examination of leucocyte entrapment

Under light microscopy, the number of segmented neutrophilic granulocytes, band neutrophils, monocytes and eosinophilic and basophilic granulocytes was significantly reduced along the flow direction through the three layers of the cross-section of the filter material (table 3).

The middle and last blood contact layer showed statistically significantly fewer granulocytes than the first layer ($p < 0.0001$). Lymphocyte counts, however, did not differ significantly between the first two layers but were significantly reduced in the last layer (table 3). In total over 3 layers, granulocytes comprised 84% of the total microscopically counted leucocytes. Lymphocytes attributed 14% to the total counted leucocytes. The electronically measured composition of leucocytes in blood before filtration was 78% granulocytes and 22% lymphocytes. Massive adhesion of granulocytes to the filter material was also shown by scanning electron microscopical pictures of the filter material after use in the clinic (figure 1). Additional electronic leucocyte counting in blood revealed an average leucocyte removal during the first 10

Table 3. Different microscopic leucocyte counts in 3 layers of the cross-section of the filter material.

	*count in cross-section**			*P value*
	1	*2*	*3*	
segmented neutrophils	294.5 ± 23.29	61.5 ± 21.92	26.2 ± 10.69	0.0001
band neutrophils	13.0 ± 1.88	3.3 ±1.63	1.3 ± 0.81	0.0001
basophilic granulocytes	1.6 ± 0.33	0.3 ± 0.15	0.1 ± 0.07	0.0001
eosinophilic granulocytes	11.4 ± 2.27	1.0 ± 0.53	0.2 ± 0.12	0.0001
lymphocytes	22.4 ± 2.47	28.2 ± 2.25	17.5 ± 2.51	0.0158†
monocytes	8.5 ± 1.00	1.7 ± 0.63	0.7 ± 0.35	0.0001

Each layer was counted in total 102 times in 9 patients (n = 9). Counts are expressed as mean and standard error per layer. *P*-values indicate difference between the 3 layers after Duncan's post hoc testing.
Layers: 1, first blood contact layer; 2, middle layer; 3, last blood contact layer; †, significance was found only between the middle and last layer

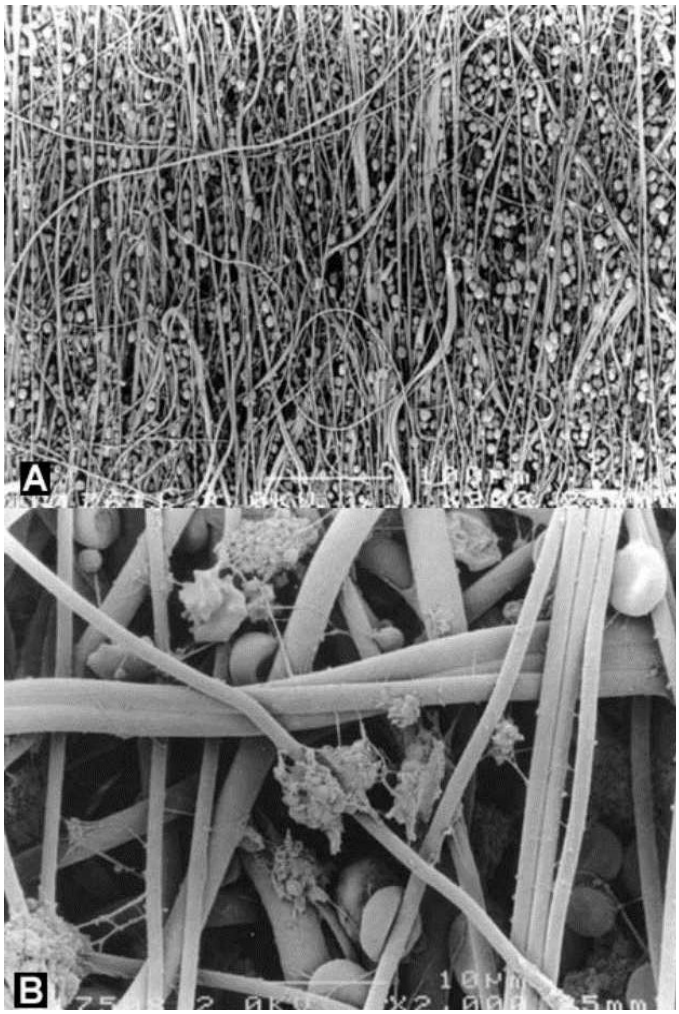

Figure 1. Electron microscopic pictures showing the filter material and trapped cells from a patient who received filtration (**A** magnification x290, **B** magnification x2900). Granule-rich neutrophils are clearly distinct from the red blood cells after the dehydration process in preparation for electron microscopy.

Table 4. Electronically measured leucocyte, granulocyte and lymphocyte removal

time points (min)	unit	leucocytes	granulocytes	lymphocytes
2	%	90.6 ± 3.0	92.7 ± 3.4	89.9 ± 3.5
4	%	83.1 ± 5.5	87.1 ± 6.6	79.9 ± 4.2
6	%	78.0 ± 6.9	82.5 ± 8.0	72.6 ± 5.5
8	%	71.5 ± 8.5	78.3 ± 9.8	64.9 ± 6.3
10	%	69.7 ± 8.4	75.3 ± 9.3	56.9 ± 8.4
0 - 10	%	79.0 ± 4.0	83.5 ± 3.6	73.4 ± 6.6
0 - 10	10^9 / 5 L	12.6 (4.2 - 19.4)	9.8 (4.9 - 16.4)	3.0 (1.1 - 4.7)

Values are expressed as mean ± standard error, in percentage of the cell count measured before and after the filter, at 5 different time points during filtration ($n = 11$). Additionally, the average removal of leucocytes during 10 minutes filtration in percentage (mean ± standard error) and the total absolute number (10^9 / 5 L blood) of removed leucocytes (number + range) in 10 minutes, are shown

minutes of filtration of 79% (table 4). Granulocyte removal was 84% and lymphocyte removal 73% (table 4). In five sequential measurements during the first 10 minutes a gradual decrease of leucocyte removal, from 91% to 70% (table 4), was observed. However, in spite of 10 minutes leucocyte filtration, the average systemic leucocyte counts measured in 11 patients did not change during the period of filtration (before filtration 3.68 ± 0.42, after filtration 3.62 ± 0.49; *p*-value 0.94).

Immunologic examination of activated granulocyte entrapment

A significant increase in CD45RO binding to the filter material after leucocyte filtration in comparison with the non-specific binding without leucocytes was found ($P<0.001$, figure 2). In the middle layer no increase was found in the CD45RO expression after further stimulation of granulocytes with Zymosan-activated plasma (figure 2). In the first and last layers, however, CD45RO expression increased after Zymosan stimulation (figure 2). Microscopic granulocyte count, CD45RO binding, and maximal CD45RO binding to the filter decreased significantly along the flow direction. Proving the validity of the test, the decrease of microscopical granulocyte counts correlated best with the decrease in maximal CD45RO expression. A significant CD2 binding (T cells) to the three different filter layers as compared with the non-specific binding was discovered. CD19 binding (B cells), however, was significantly different only from the non-specific binding in the middle layer. The average CD2 binding was, in accordance to the microscopic lymphocyte count, highest in the middle layer, although no significant difference was found between the layers (figure 3).

The additional antibody tests by means of flowcytometric assessment on whole blood samples showed a clearly detectable CD11b expression in blood before filtration, whereas after filtration the CD11b expression was below the detection limit. The

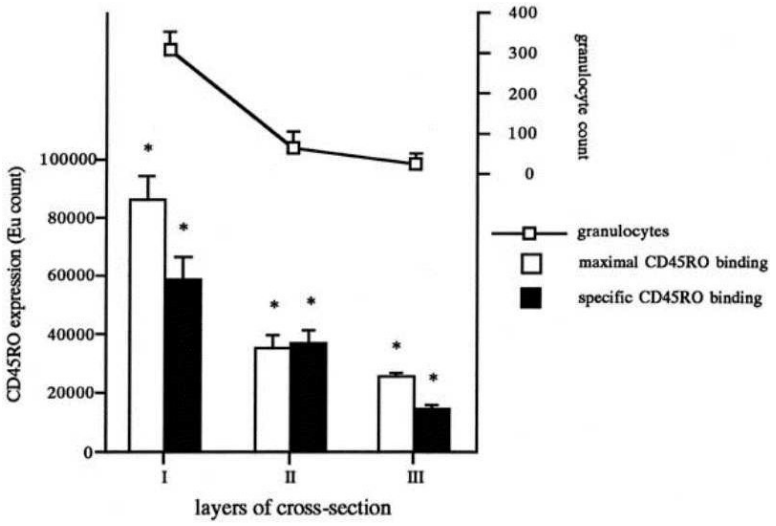

Figure 2. Granulocyte and CD45RO binding to the filter material in 3 different layers. I, first blood contact layer; II, middle layer; III, last blood contact layer. Specific CD45RO binding (calculated as CD45RO binding minus the non-specific binding) to the filter material is shown in black. Maximal CD45RO binding (after activation of the cells with zymosan-activated plasma) (white bars) was higher in the first and third layer than the specific binding without stimulation. The specific binding was significantly different from the non-specific binding (25,000 ± 6000, not shown). Granulocytes were counted in one microscopic field.

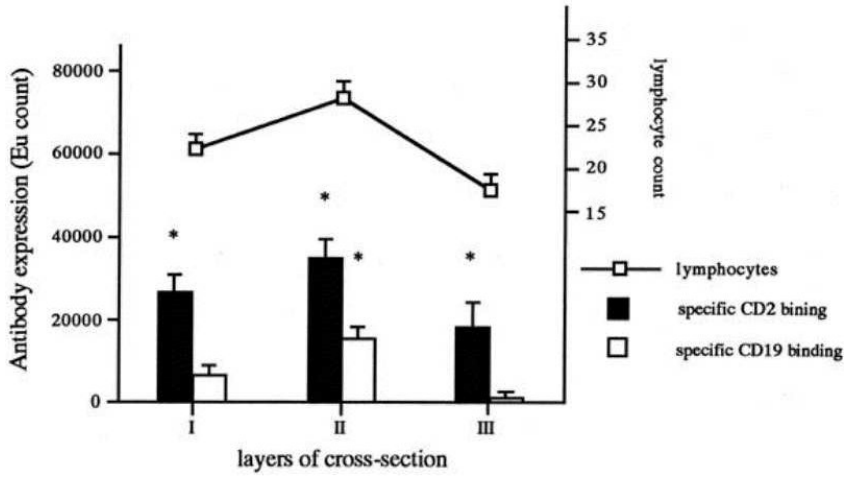

Figure 3. Lymphocyte and CD2 and CD19 binding to the filter material in 3 different layers. I, first blood contact layer; II, middle layer; III, last blood contact layer. Specific CD2 binding to the filter material (calculated as CD2 binding minus the non-specific binding: 57,000 ± 2000, not shown) was significantly increased as compared with the non-specific binding (*$p < 0.01$). CD19 binding (calculated as CD19 binding minus the non-specific binding: 73,000 ± 2000, not shown), was significantly increased in the middle layer (*$p < 0.05$). Lymphocytes were counted in one microscopic field.

removal of CD11b expressing cells, however, did not result in a significant reduction of the CD11b expression of leucocytes remaining in the systemic circulation after filtration. The background count, measured by a control antibody remained during the whole procedure less than 4% of the initial count before filtration.

Other biochemical measurements in blood samples

ß-glucuronidase, measured in 8 patients, was 0.17 ± 0.02 before the filter and 0.17 ± 0.03 after the filter (p = 0.82), indicating no activation of granulocytes by the filter material or of granulocytes attached to the filter material. Furthermore, PAF-IC was not reduced after the filter as compared with before the filter.

DISCUSSION

The removal of leucocytes during CPB has been reported to reduce postoperative complications after heart surgery.[12,13,21] It is not known, however, whether this reduction is due to the removal of activated granulocytes or to at-random removal of leucocytes. This study showed that the filter removed mainly the activated granulocytes, as indicated by the following findings. First, CD45RO, a specific marker for activated granulocytes showed a significant and almost maximum amount of binding to the filter material, indicating that the majority of granulocytes trapped inside the filter material was activated. Second, a nearly complete reduction of CD11b, another marker of activated granulocytes, after the filter suggests the removal of activated granulocytes. Third, the granulocyte portion of total leucocytes inside the filter material was 84%, as compared to 77% in blood before filtration. The lymphocyte portion inside the filter material was 14% compared with 23% in the blood before filtration. Therefore the filter material trapped primarily granulocytes rather than lymphocytes.

The enormous CD45RO binding to the filter material in the first layer, where 75% of the granulocytes were trapped, was followed by a maximum CD45RO expression level in the middle layer. However, CD45RO binding to the middle layer might have been caused by the enhanced portion of lymphocytes. The last layer, in contrast, functioned less efficiently in respect to the amount of bound cells and the activation level. However, the CD45RO counts per granulocyte in the last layer were approximately two times higher than those in the first layer. The amount of CD45RO binding in the last layer might also have been enhanced by a relative increase in the percentage of lymphocytes (38% in the last layer as compared with 6% in the first layer). Absolute or relative enhancement by lymphocytes in the middle and last layer may therefore have been the cause of the discrepancy between the microscopically counted granulocytes and the CD45RO binding. The filter was composed of one layer that we artificially divided into three layers. Eliminating the last blood contact layer may have reduced the loss of lymphocytes. In patients undergoing CPB, lymphocytes are preferably preserved to maintain the host defence mechanism. Clearly a less-solid structure of the filter material would reduce the potential danger of flow obstruction by filter resistance. Even in a filter with a large pore size such as the one we used (figure 1), the accumulation of leucocytes might activate other blood elements, resulting in a cascade reaction that could obstruct the filter and damage the blood. The release of PAF might play a crucial role in activating platelets as the origin of a large clotting reaction.[22,23] PAF levels caused by

this filter, fortunately, were not enhanced after filtration. Also coagulation may be enhanced by Factor X binding to CD11b, which is clearly expressed on the granulocytes during CPB.[24]

Although CD45RO was originally used for detecting 45% of T and a few B cells, it was lately discovered that the antibody also binds to activated granulocytes.[25-29] Since the filter material was especially designed for binding granulocytes, the expectation was that CD45RO would primarily be a marker for activated granulocytes. Given the results of the CD2, CD19 and microscopic leucocyte count in the filter material, it can also be concluded that CD45RO primarily measured the binding of activated granulocytes. CD2 binding to the filter material was not extensive and did not significantly differ among the three layers of the filter material. CD19, reflecting B cell binding did not even bind to the first and last blood contact layer. CD45RO, on the contrary, bound massively in accordance with the amount of microscopically counted granulocytes to the first layer and decreased in average in the middle and last layers. Therefore it can be concluded that this massive CD45RO binding to the first layer was at most 10% caused by the binding of lymphocytes. Furthermore, CD45RO is thought to be suitable marker for activated granulocytes, although this is not widely accepted.

The microscopic leucocyte counts of the filter material were also useful as controls for the antibody tests. In support of the validity of the antibody tests, and the CD2 and CD19 binding tests, the microscopical leucocyte counting tests showed a slight increased average binding of lymphocytes to the middle layer of the filter material. In addition, at a maximum CD45RO expression level of the granulocytes, Eu counts correlated perfectly with the number of microscopically counted granulocytes.

The leucocyte removal rate from circulating blood declined from 90% to 70% at the end of filtration, although large individual differences in the absolute numbers of removed cells existed among the patients (4.2 to19.4 x 10^9 / 5 liters blood). Therefore it is less likely that the filter lacked capacity for leucocyte adhesion; otherwise all patients would have demonstrated a similar total amount of removed leucocytes. A more plausible explanation would be the internalization of adhesion receptors by the filter material after the first pass, resulting in a decreased adhesive capacity of leucocytes contacting the filter material for the second time. The failure of leucocytes to adhere to artificial surfaces when exposed to monoclonal antibodies against CD11b receptors has been described.[30] Also, the internalization of CD11b receptors after exposure to various stimuli has been reported.[31-33] Thus it might be more wise to enlarge the first contact layer of leucocyte filters instead of the thickness of leucocyte filter material.

Although a considerable amount of activated granulocytes have been trapped in the filter, the systemic granulocyte counts did not change during the period of filtration. This is most likely due to the fact that the systemic rewarming of blood started in parallel with leucocyte filtration, which means that a new population of granulocytes entered the blood stream massively from the third space, extravascularly, and from the bone marrow. Other evidence supporting leucocyte entry into the blood stream was the finding of level amounts of granulocytes expressing CD11b in the pre-filter blood samples during the whole period of filtration, in spite of a clear removal of CD11b expressing cells. Actually, this newly released population of granulocytes in the circulation counteracts the reduction effect made by leucocyte filtration. Indeed, the reduction of systemic leucocytes by leucocyte filtration is obvious when compared with results in a group of

control patients whose increase in systemic leucocytes during the rewarming phase was more severe than in filtration patients.

It seems that the adhesion of granulocytes to the filter material did not result in further granulocyte activation, since there was no enhanced ß-glucuronidase, and PAF from stored granules. It demonstrates that the cellular structure remains intact during the relatively mild flow conditions in our venous bypass circuit.

In conclusion, we have shown that the activated part of the granulocytes and not leucocytes at random are removed by the presented type of leucocyte filter. These leucocyte removal filters may appear suitable for use in heart operation patients, whose granulocytes in the circulation are activated. Individual differences in granulocyte activation level might therefore be a plausible explanation for the large differences in filtration efficiency between patients. Moreover, the measurement of activated granulocytes instead of total leucocyte count might be preferable for assessment of leucocyte filter devices in the future. Furthermore, this study shows that the filter likely does not need a high capacity, since the activated granulocytes either adhere immediately or do not adhere at all. Finally, removal of activated granulocytes during CPB did not alleviate the patient's exposure to activated granulocytes, since the remaining granulocytes and those from the marginating pool replaced the removed cells immediately. Therefore, to reduce the post-perfusion syndrome, repeated leucocyte filtration should be considered to enhance the efficiency of filtration.

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CHAPTER 5

LEUCOCYTE DEPLETION DURING CARDIAC SURGERY: A COMPARISON OF DIFFERENT FILTRATION STRATEGIES.

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ABSTRACT

The results of leucocyte filtration during cardiac surgery are conflicting. This may be due to timing and duration of the filtration procedure, and to flow and pressure conditions in the filter. Therefore, we prospectively compared three major leucocyte filtration strategies in cardiac surgical patients.

Forty patients were randomly divided into four groups. Group I: leucofiltration of arterial blood throughout cardiopulmonary bypass (associated with high flow and pressure gradients), group II: Leucofiltration of a part of the venous return blood in the rewarming phase during cardiopulmonary bypass (associated with intermediate flow, but high pressure), group III: Leucofiltration of residual heart-lung machine blood during transfusion into the patient after cardiopulmonary bypass (associated with low flow and low pressure), group IV: Control group without leucofiltration. We measured circulating leucocyte counts, plasma elastase levels and arterial blood oxygenation. Filters were postoperatively examined by scanning electronmicroscopy.

We found that leucocyte counts increased over time and oxygenation decreased in all groups, without significant difference between the groups. Scanning electron-microscopy demonstrated extensive protein deposits and damaged leucocytes in the deeper layers of the filters from group I. This was not observed in the filters from group III. The postoperative plasma elastase levels increased in group II and IV and decreased in group I and III.

The results from this study did not demonstrate a clinical difference among the three leucocyte depletion strategies. However, our laboratory results suggest that leucocyte filtration at low flow and pressure conditions is associated with less leucocyte damage and less release of elastase.

INTRODUCTION

Cardiopulmonary bypass (CPB) leads to a well known systemic inflammatory response and contributes to postoperative morbidity and organ dysfunction.[1-3] Polymorphonuclear leucocytes and complement are activated as a result of blood contact with the surface of the CPB-circuit and are considered as important causes for organ dysfunction, notably of the lungs.[4-6] Leucocyte depletion by means of filtration has been introduced into clinical practice to reduce this inflammatory response. However, the reported clinical results of leucofiltration are conflicting. Some studies demonstrate a reduction in leucocyte counts[7-13] and an improvement in ventilatory parameters,[7-8] whereas others do not.[14-17] These differences may be caused by the timing and duration of the filtration procedure during the operation, and the location of the filter in the CPB circuit. For instance, filtration may be applied throughout CPB,[7.10,13-15] or in short, but well aimed time spans;[9,12,16] on the arterial,[7,9,10,12-15] or venous[11] side of the CPB circuit; or even outside the CPB circuit, as for filtration of residual heart-lung machine blood.[8] In addition, these various filtration procedures are associated with different flow and pressure conditions over the filter. Filters used under high pressure and flow conditions (e.g. arterial line filters), may have other characteristics of leucocyte entrapment than filters used under low pressure and flow conditions (e.g. filters for residual heart-lung machine blood). It is not known how these factors affect the filter efficiency.

Therefore, we compared in this study the clinical effects of leucofiltration via the arterial line throughout CPB, leucofiltration via the venous line during rewarming and leucofiltration of residual heart-lung machine blood after CPB. These are the three major strategies for leucofiltration during cardiac surgery, each associated with a different volume filtered and with different flow and pressure conditions. In an attempt to study the effects of flow and pressure conditions during filtration, we also examined filters after use with scanning electron microscopy (SEM).

MATERIALS AND METHODS

Patients and filtration procedures

After institutional human investigation committee approval and patient consent, 40 patients scheduled for elective coronary artery bypass grafting (CABG) or valve replacement were randomly allocated to 4 groups of 10 patients each. Exclusion criteria were pre-existing lung disease, emergency operation and re-operation. In group I, leucofiltration was achieved throughout cardiopulmonary bypass (CPB), using a high flow leucocyte removal filter (LG6, Pall Biomedical, Portsmouth, UK), incorporated in the arterial line. This procedure was associated with high flow and pressure gradients over the filter. In group II, leucofiltration of a part of the venous return blood was achieved during CPB in the rewarming phase until aortic cross-clamp release, using paired leucocyte removal filters (RS 1, Pall Biomedical, Portsmouth, UK) as previously described.[11] Blood flow was adjusted with a separate roller pump to 400 mL/min. The filtration pressure, measured between the pump and the filter, was generally high, ≥ 150 mmHg, but did not exceed 300 mmHg. The filtration procedure

lasted 10 ± 0.7 min., and thus the amount filtered was 4000 ± 80 mL. This procedure was associated with intermediate flow, but high pressures. In group III, leucofiltration of the residual heart-lung machine blood (1.2 to 2 L) was achieved as it was transfused into the patient after CPB, using a leucocyte removal filter (RS 1, Pall Biomedical, Portsmouth, UK). The blood was transfused under gravity, ≤ 100 mmHg. This procedure was associated with low flow and low pressures. In group IV, no leucofiltration was applied. These patients served as controls.

Methods

Anaesthesia was induced and maintained by intravenous infusion of sufentanil (1 to 3 μg/kg) and midazolam (0.05 to 0.1 mg/kg). Pancuronium (0.1 mg/kg) was used for muscle relaxation. Ventilatory management was aimed at normocapnia throughout the operation and in the intensive care unit (ICU), with an inspiratory oxygen fraction of 0.4, a positive end-expiratory pressure of 6-8 cm H_2O and a tidal volume of 6-8 mL/kg. Dexamethasone (1 mg/kg) was administered after induction. Bovine lung heparin (300 IU/kg) was used for anticoagulation. This was monitored by the celite activated clotting time (International Technidyne Co., Edison, N.J., USA) and maintained at a value of at least 400 s. After CPB, heparin was neutralized by protamine (300 IU/kg).

The extracorporeal circuit consisted of roller pumps (Stöckert Istrumente GmbH, München, Germany) and a membrane oxygenator (CML Excel, Cobe Laboratories, Lakewood, CO, USA) primed with 500 mL hydroxyethylstarch 10% (Haes, Fresenius, Bad Homburg, Germany) and 1500 mL lactated Ringer's solution. Arterial line filters, other than the one studied, were not used. Flow rate was adjusted to 2.4 $L/m^2/min$. Blood pressure during CPB was kept between 50 and 80 mmHg and nasopharyngeal temperature was maintained at 30°C. The surgical wound suction blood was returned to the cardiotomy reservoir of the CPB circuit in all patients. Cell-savers were not used. The residual heart-lung machine blood after CPB (± 1.3 L) was transfused into the patients in all groups.

Scanning electronmicroscopy

Histological examination of the leucocyte filters by SEM was performed as previously described.[18] Briefly, 3 filters in each group were after filtration rinsed with 500 mL normal saline by a roller pump at a flow rate of 100 mL/min. After rinsing each filter was opened and two samples of the filter medium were taken. Each sample was divided in three layers, a superficial layer where the blood entered the filter, a middle layer and a deep layer where the blood left the filter. All samples were immediately fixated in a 2% glutaraldehyde solution with 0.1 M cacodylate buffer at pH 7.4. Further processing consisted of standard SEM preparation, including fixation with 1% osmium-tetra-oxyde, dehydration in ethanol series, critical point drying and gold sputter coating. The samples of the three layers were subsequently studied with SEM (JEOL 6301F, Tokio, Japan) by two independent observers to obtain a qualitative assessment of the filter characteristics.

Clinical Measurements

Blood samples for laboratory tests and biochemical assays were drawn from the radial artery of the patient after induction of anaesthesia, at the end of the operation, after 3 hours in the ICU and on the morning of the first postoperative day. The arterial oxygen tension (PaO_2) was measured and the alveolar-arterial (A-a) oxygen gradient was calculated, using standard formulae. From EDTA-anticoagulated blood, haematocrit and platelet, total white blood cell and granulocyte counts were determined by an electronic cell counter (Cell-Dyn 610, Abbott, Santa Clara, CA, USA). Plasma elastase, as marker of leucocyte activation, was determined using an enzyme immunoassay (Merck, Darmstadt, Germany).

Perioperative fluid balance, use of inotropic agents, myocardial infarctions (defined as new Q-wave on the ECG and CK > 180 U/L with CK-MB > 10% of total), of postoperative intubation, and length of stay in the ICU and the hospital were recorded from the patient charts. The attending ICU and hospital staff were blinded to the study group.

Statistics

All data are presented uncorrected for haemodilution and expressed as mean ± standard error, except for elastase for which percentages are used, because of a high range of starting values. For comparison between the groups one way analysis of variance (ANOVA) was used with a post-hoc analysis using the Bonferroni method when necessary. To determine the effects of time and interaction between the groups over the different time points repeated measurements ANOVA was used. To correct for non-sphericity Greenhouse-Geisser (ε) adjustments were made. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Clinical course

The patient groups were similar with respect to the demographic data (table 1). The CABG patients typically had 3 grafts of which at least one arterial graft. Intubation times, length of stay in the ICU and the hospital, use of inotropes (dopamine, 5-8 μg/kg/min), postoperative blood loss and the overall postoperative fluid balance were similar among the patient groups (table 2). One patient in group I had a myocardial infarction. Two patients died: one patient in the control group IV who developed a low output state and respiratory insufficiency, and one patient in the arterial group I who had a massive gastro-intestinal bleeding on the ward.

The PaO_2 decreased in the 4 groups over the different time points with a significant time effect ($p < 0.001$). Although the residual group III had higher postoperative mean values than the other groups, a significant group effect was not present ($p = 0.53$, figure 1). The PaO_2 values on the first postoperative day were similar in all groups (table 2). The postoperative A-a gradients increased with a significant time effect ($p < 0.001$). Although group III had the lowest mean values, a significant group effect was not present ($p = 0.62$, figure 1). The A-a gradients on the first postoperative day were similar in all groups (table 2).

Table 1. Patient characteristics

Group	Arterial (I)	Venous (II)	Residual (III)	Contol (IV)	*p*-value
Age	68.4 ± 2.6	66.2 ± 2	61.4 ± 2.5	66.9 ± 3.4	0.30
Height (cm)	170 ± 2.5	170 ± 1.5	171 ± 2.9	171 ± 2.3	0.97
Weight (kg)	80.4 ± 2.7	72.1 ± 2.3	75.7 ± 4	75.6 ± 3.6	0.35
Male (n)	6	7	6	6	
Valve (n)	3	3	2	4	
CPB time (min)	126 ± 18.3	14 ± 0.110 ± 9.9	99 ± 8.5	113 ± 17.1	0.62
Filtrate (l)	598 ± 95		1.7 ± 0.1	0	< 0.001

Filtrate in the arterial group I was calculated from CPB time and a pump flow of 2.4 $l.min^{-1}.m^{-2}$. Filtrate in the venous group II was calculated from start rewarming to aortic cross-clamp release (= 10 ± 0.7 min). Data are shown as mean ± standard error; one way analysis of variance was used for statistical analysis.

Table 2. Postoperative data

Group	Arterial (I)	Venous (II)	Residual (III)	Control (IV)	*p*-value
WBC-day1 ($x10^9.l^{-1}$)	11.5 ± 0.6(10.1-13)	16.6 ± 1.3(13.5-19.7)	13.1 ± 0.8(11.3-15)	14.4 ± 1.4(11.3-17.5)	0.04
PMN-day1 ($x10^9.l^{-1}$)	10.9 ± 1.2(8.5-13.3)	14.7 ± 1.2(12.2-17.3)	12 ± 1(9.9-14.2)	14 ± 1.2(11.6-16.4)	0.03
PaO_2-day1 (kPa)	13.2 ± 1.2(10.6-15.8)	11.3 ± 1(9-13.6)	13.8 ± 1.2(11.1-16.5)	13.2 ± 0.9(11.2-15.3)	0.47
A-a grad-day1 (kPa)	18.8 ± 1.2(16.1-21.5)	21.4 ± 1(19-23.7)	18.5 ± 1.2(15.8-21.	18.7 ± 0.8(16.9-20.6)	0.31
Intubation (h)	26.1 ± 10.6(2.1-50.1)	12.5 ± 1.8(8.4-16.6)	13 ± 1.5(9.5-16.5)	25.2 ± 11.2(-0.5-50.5)	0.44
Fluid balance (ml)	-146 ± 408(-1070-778)	-13 ± 456(-1064-1038)	412 ± 444(-593-1418)	899 ± 330(153-1645)	0.33
Blood loss (ml)	705 ± 159(344-1067)	751 ± 117(480-1021)	676 ± 164(304-1048)	735 ± 154(387-1084)	0.98
ICU stay (hours)	35.7 ± 11.8(9-62)	28.1 ± 3.4(20.5-35.7)	23.4 ± 0.6(22-24.9)	34.9 ± 12.1(7.5-62.4)	0.71
Hospital stay (days)	12.5 ± 3(5.6-19.4)	9.1 ± 1.5(5.6-12.6)	7.8 ± 0.4(6.8-8.8)	12.3 ± 2.4(7-17-6)	0.30

WBC-day1, circulating leucocyte count on the first postoperative day; PMN-day1, circulating granulocyte count on the first post-operative day; PaO_2-day 1, arterial oxygen tension on the morning of the first postoperative day; A-a grad-day1, alveolar-arterial oxygen gradient on the first postoperative day; intubation, duration of postoperative ventilation; fluid balance, difference in total fluid put and total fluid output during the first 24 hours; blood loss, chest tube drainage in the first 24 hours. Data are shown as mean ± standard error and beween brackets the 95% confidence limits; one way analysis of variance was used for statistical analysis.

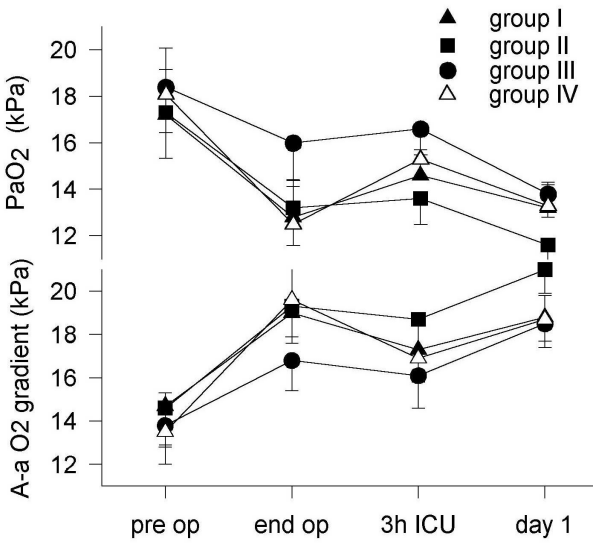

Figure 1. Pre- and postoperative arterial oxygen tension (PaO_2) and alveolar-arterial (A-a) oxygen gradients in the three leucocyte filtration groups and in the unfiltered control group at an inspiratory oxygen fraction of 0.4. Pre op, preoperatively; end op, at the end of operation; 3h ICU, at 3 hours in the intensive care unit; day 1, the morning of the first postoperative day; group I, leucofiltration of arterial blood throughout cardiopulmonary bypass; group II, leucofiltration of a part of the venous return blood during rewarming; group III, leucofiltration of residual heart-lung machine blood after cardiopulmonary bypass; group IV, controls without leucofiltration. Values shown are the means, estimated by the repeated measurement model with standard error. The PaO_2 showed a significant decrease over time ($p < 0.001$), but no group effect by repeated measurements analysis of variance. The A-a gradients showed a significant increase over time ($p < 0.001$), but no group effect by repeated measurements analysis of variance.

Haematology and biochemistry.

The leucocyte counts increased in all groups from the end of CPB towards the first postoperative day with a significant time effect ($p < 0.001$). There was no difference between the groups ($p = 0.91$). The lowest leucocyte counts on the first postoperative day were observed in the residual group III in the arterial group I. There was a significant difference between the leucocyte counts in group I and the venous group II on the first postoperative day (table 2, figure 2). The granulocyte counts also increased, showing a significant time effect ($p < 0.001$), but no difference between the groups ($p = 0.07$). There was a significant difference between the granulocyte counts in group I and group II on the first postoperative day (table 2, figure 2). Analysis of the results with the type of operation, i.e. CABG or valve replacement, as a cofactor revealed a significant ($p = 0.04$) effect on the leucocyte and granulocyte counts. The platelet

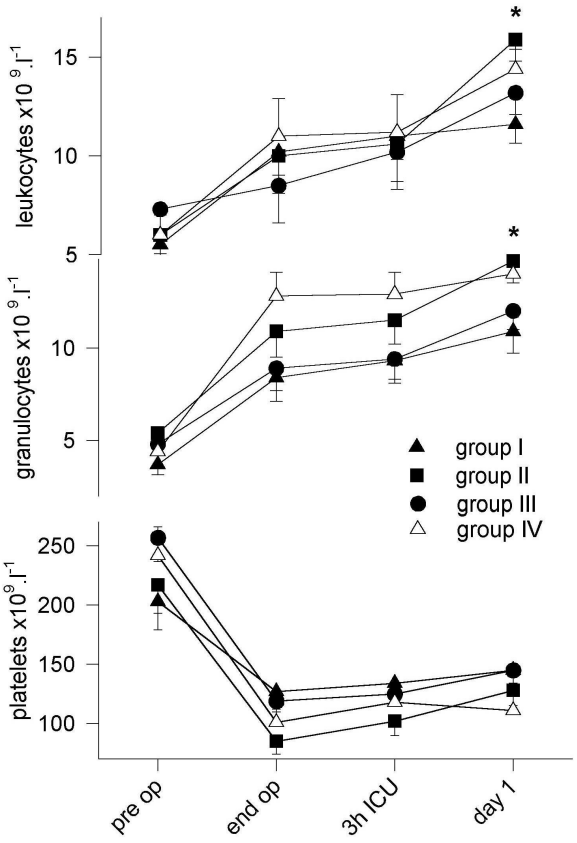

Figure 2. Pre- and postoperative circulating leucocyte, granulocyte and platelet counts in the three leucofiltration groups and in the unfiltered control group. Pre op, preoperative-ly; end op, at the end of operation; 3h ICU, at 3 hours in the intensive care unit; day 1, the morning of the first postoperative day; group I, leucofiltration of arterial blood throughout cardiopulmonary bypass; group II, leucofiltration of a part of the venous return blood during rewarming; group III, leucofiltration of residual heart-lung machine blood after cardiopulmonary bypass; group IV, controls without leucofiltration. Values shown are the means, estimated by the repeated measurement model with standard error. Leucocyte and granulocyte counts showed a significant increase over time ($p < 0.001$), but no group effect by repeated measurements analysis of variance. Platelet counts showed a significant decrease over time ($p < 0.001$), but no group effect by repeated measurements analysis of variance. * $p < 0.05$ between group I and group II by analysis of variance.

counts decreased at the end of the operation and then gradually increased, showing a significant time effect ($p < 0.001$), but no difference between the groups ($p = 0.49$). The elastase measurements showed two distinct patterns because a significant group interaction was present ($p = 0.005$) (figure 3). At the end of the operation, the elastase values were increased in the arterial group I ($p = 0.03$). After the operation, the elastase values increased in the control group IV and venous group II. Repeated measurements ANOVA revealed a significant time effect ($p = 0.02$), but no group effect ($p = 0.54$).

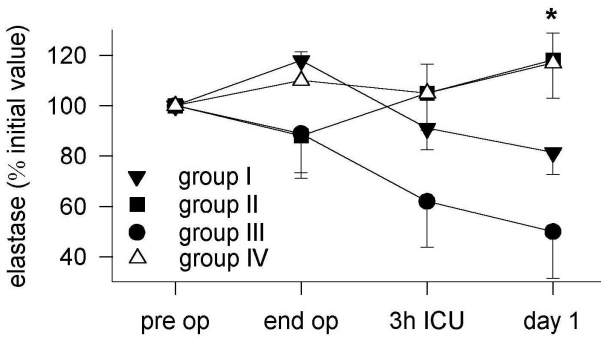

Figure 3. Pre- and postoperative plasma elastase levels in the three leucofiltration groups and in the unfiltered control group. Pre op = preoperatively, end op = at the end of operation, 3h ICU = at 3 hours in the intensive care unit, day 1 = the morning of the first postoperative day. Group I: leucofiltration of arterial blood throughout cardiopulmonary bypass; Group II: leucofiltration of a part of the venous return blood during rewarming; Group III: leucofiltration of residual heart-lung machine blood after cardiopulmonary bypass; Group IV: controls without leucofiltration. Values given are percentages of starting values which were: group I 230 ± 42 µg/L, group II 74 ± 6 µg/L, group III 29 7 ±45 µg/L and group IV 96 ± 21 µg/L. Repeated measurements analysis of variance revealed a significant time/group interaction ($p < 0.001$), indicating two distinct patterns. * $p < 0.05$ between group II and group III by analysis of variance.

The elastase values decreased after the operation in the residual group III and arterial group I. Analysis revealed a significant time effect ($p = 0.01$), but no group effect ($p = 0.61$). There was a significant difference in elastase levels on the first postoperative day between group II and group III ($p = 0.03$) and between group III and group IV ($p = 0.04$).

Scanning electronmicroscopy.

The arterial filter (group I) had fibres of about 15 µm diameter and wide interspaces of about 70 µm. The filter had a three-dimensional structure based on a mesh of single fibres. Extensive platelet and protein deposits almost completely covered the filter fibres and trapped cells (figure 4, a1). Many platelets, but very few leucocytes were trapped in the superficial layer of the filter. In the two deeper layers of the filter many leucocytes and red blood cells were present, often damaged as shown by the rough, irregular shape (figure 4, a2 middle). Some red blood cells were caught in the protein network. Platelets had pseudopodia indicating activation (figure 4, a2). Platelet deposition decreased in the deeper layers of the filter.

The venous (group II) and residual (group III) filters had fibres of about 3 µm diameter with narrow interspaces of about 10 µm. These filters had a similar three-dimensional structure as the arterial filter. Two distinct cellular patterns were observed, depending on the pressure applied. In the filters used under high pressure, extended protein deposits were seen including fibrin networks (figure 4, b1). Leuco-

cytes were mainly trapped in the middle and lower layer of the filter, where also some damaged leucocytes were present (figure 4, b2 and b3). The platelets had a predominantly rounded appearance (figure 4, b2), but in the superficial layer, many platelets had pseudopodia (figure 4, b1). In contrast, in the filters used under low pressure, leucocytes and platelets were predominantly located in the superficial filter layer. The leucocyte and platelet entrapment was grossly reduced in the middle layer. In the lower layer hardly any leucocytes and platelets were seen (figure 4c). There were virtually no protein deposits. Only the platelets in the superficial layer had pseudopodia. Leucocytes were in excess of the platelets.

DISCUSSION

In the cardiac surgical patients studied, we did not find a clinical difference among the three filtration groups. Furthermore, there was no clinical difference between any of the filtration groups and the control group, where no leucocyte filtration was applied. As such, this study is essentially a negative one with respect to leucocyte filtration. However, the finding of different patterns of leucocyte enptrapment with different pressure and flow conditions is new and may explain some of the controversies that exist about clinical leucocyte filtration.

The first leucocyte filtration strategy for cardiac surgical patients, which is currently most common, is an arterial line filter used throughout CPB. However, the effects on leucocyte counts and PaO_2 resulting from this approach are conflicting.[7,14] We could not demonstrate a beneficial effect of arterial line filtration on postoperative leucocyte counts, PaO_2 and A-a gradients in this study. These findings are supported by others.[10,14,15] At least two explanations can be found in the interpretation of the SEM data. First, the leucocytes that are bound in the arterial line filter remain in the circulation and thus are subjected to the high pressures, up to 200 mmHg, and high flows, of 4-5 L/min, generated in the CPB circuit. The SEM data showed extensive protein deposits, and leucocytes that were pressed into the middle and lower filter layer. These pressure and flow conditions can also explain the damaged leucocytes that we found on SEM, and the increased elastase levels that we found at the end of CPB. This is in agreement with Mihaljevic et al. who measured the elastase levels before and after the arterial line filter and found an increase after the filter[14] and with Mair et al. who also found increased elastase levels at the end of CPB in their filter group.[10] Second, despite the large blood volume filtered, the leucocyte counts at the end of the operation were similar to the leucocyte counts in the other groups, indicating that the arterial line filter had a low efficiency and efficacy. Again, an explanation may be found in the SEM data, which show a filter with thick, wide spaced fibres. Moreover, the short contact time between leucocyte and filter material, caused by the high blood flow, may play a role as well, since an increase in contact time between leucocytes and filter material improves filtration efficiency.[18]

In the second strategy the filter was placed in a side branch of the venous line of the CPB circuit in order to create low flow conditions. This approach increased the contact time between the blood and the filter to remove leucocytes more effectively

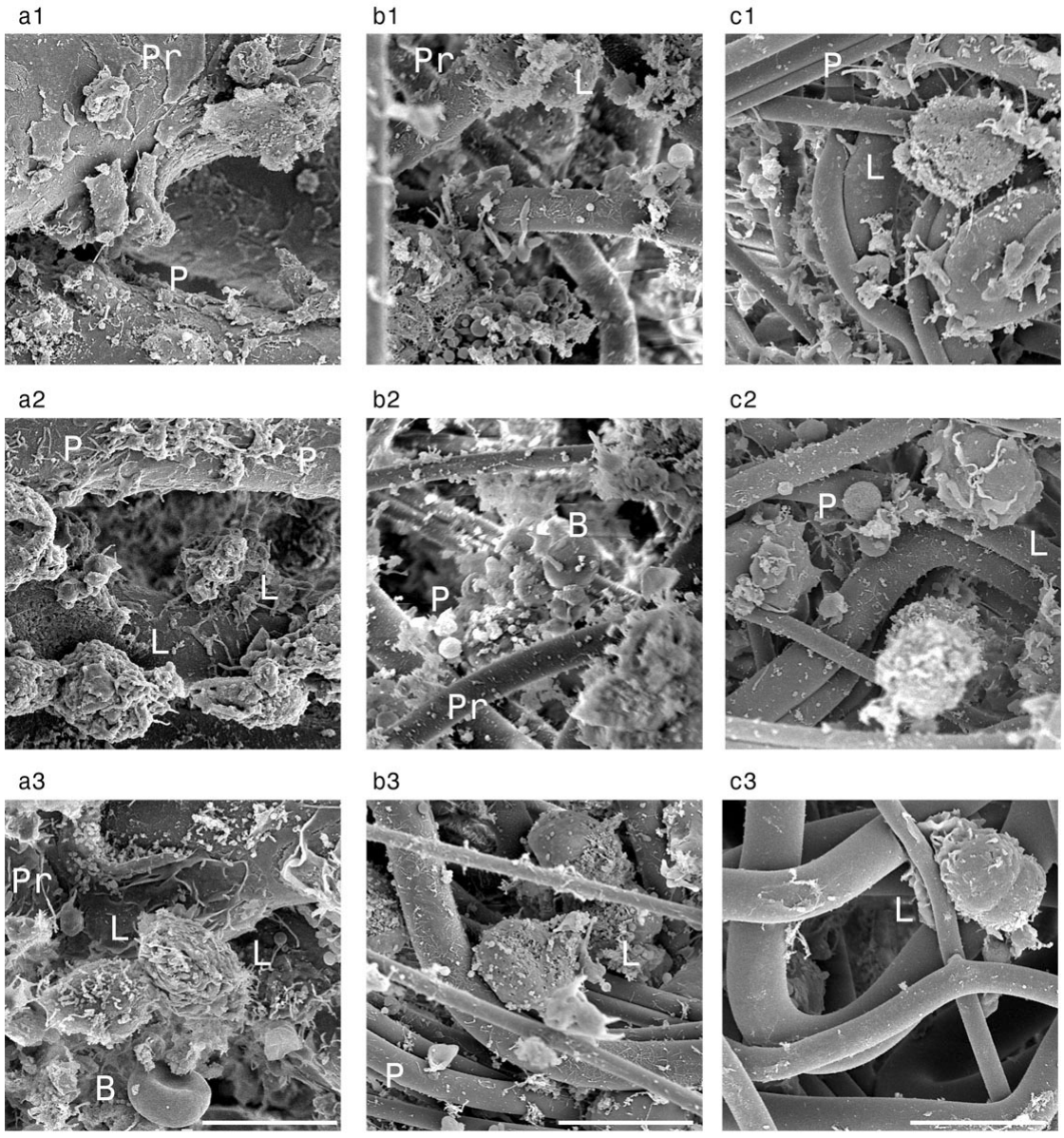

Figure 4. Scanning electronmicroscopic pictures from leucocyte filters under different pressure and flow conditions. The columns show the three different conditions as indicated. The rows show the three layers in which the filters were divided. At the top row blood entered the filter, at the bottom row blood left the filter. Note the differences in fibre thickness and interspaces between the different filters (column a vs. b and c), the extensive protein deposit in the arterial filter (column a) and the absence of cells and deposit in the low pressure venous filter (c3). The white bar in the photographs indicates 10 μm. B, red blood cell; L, leucocyte; P, platelet; Pr, protein deposit.

and had the advantage that the leucocytes caught in the filter were completely removed from the circulation after a short period of time. However, this approach did not result in clinical beneficial effects for at least two reasons. First, the procedure resulted often in high pressures due to the design of the filter circuit with paired transfusion filters. As can be seen from the SEM data, the leucocytes were pressed into the deeper filter layers as with the arterial line filters. This could explain why this filtration procedure despite the low flow resulted in high elastase levels after the operation. Second, this filtration procedure lasted only about 10 min which was probably too short to produce significant clinical effects. This is supported by the fact that this procedure resulted in high levels of circulating leucocytes and granulocytes on the first postoperative day.

The third strategy, to filter the residual heart-lung machine blood before transfusion into the patient, was based on the fact that blood that is transfused into the patient first passes the lung. The lungs are vulnerable after CPB and also have to filter the transfused, activated blood.[19] In this setting, low flow and low pressure conditions were present as the blood was transfused under gravity. Indeed, the SEM data showed undamaged leucocytes, located on the surface of the filter, and no protein deposits. This may explain the observed low elastase levels on the first postoperative day.

Two shortcomings of this study exist. The first one is related to the filters used. For safety reasons we used different filters (LG6 and RS1). This may have influenced our results, despite the fact that the chemical composition of the filter material was similar. This factor was in our opinion of less importance, as we wanted to compare the different strategies of leucofiltration which are closely linked to differences in volume filtered and type of filter used. More important however, may be the fact that after prolonged use leucocyte depletion filters become saturated as a result of massive cell deposition. For the arterial line filter this can occur after one hour of use. Despite the fact that the mean CPB times in group I as a whole were longer than that, we did not notice an extreme increase in arterial line pressure during CPB. However, we cannot exclude that the longer CPB times in group I may have influenced the results of the arterial line filtration negatively. The second shortcoming is that this study is underpowered to detect clinical differences between the groups, due to the small sample size and the fact that coronary artery surgery as well as valve operations were included. This can be deduced from the 95% confidence intervals from table 2. Most studies included only coronary artery bypass grafting. However we wanted to demonstrate effects on a mixed population as is usual clinical practice.

In conclusion, we could in this study not demonstrate a clinical difference between the filtered groups and between the filtered groups and the unfiltered control group. However, the electronmicroscopic data show that the amount of debris is a function of flow and pressure conditions. The results of this study also suggest that pressure might be more important than flow with respect to the leucocyte damage. This study also raises the question whether the filter itself caused the damaged cells, in other words made debris out of normal cells. Future larger scale studies are therefore necessary to evaluate the combined effects from different leucocyte depletion strategies on the filters and their clinical implications.

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CHAPTER 6

THE RATIONALE FOR FAT FILTRATION DURING CARDIAC SURGERY

A.J. de Vries, Y.J. Gu, W. van Oeveren

Perfusion 2002;17:29-33

ABSTRACT

Improved filter technology may enable the removal of specific substances such as lipids from the blood. Lipids form a heterogeneous group of compounds, but during surgery main interest is focussed on triglycerides, glycerol and free fatty acids. Fat emboli have been demonstrated in the brain after cardiac surgery and are associated with ischaemic brain injury. Fat emboli have also been demonstrated in lung and kidney tissue. Lung tissue and leucocytes are especially vulnerable to the effects of free fatty acids. The surgical wound suction blood during cardiac surgery contains a considerable quantity of micro emboli. Therefore, as a first step to determine the place of fat filtration during cardiac surgery, the use of a fat removal filter for surgical wound suction blood is advocated.

INTRODUCTION

From the early years of extracorporeal circulation, filtration techniques have been used during cardiac surgery to prevent the transferral of micro embolism and to prevent the deleterious effects of cardiopulmonary bypass (CPB) on the various organ systems.[1,2] Screen filters have been used in various positions in the CPB circuit. Their action is mainly depending on filter pore size. Application of these filters has lead to a reduction of mortality after cardiac surgery.[3] However, these filters were of limited use in removing fat particles due to the deformability of these particles.
Polymorphnuclear leukocytes and complement activation are known to play a central role in the development of the systemic inflammatory response and organ reperfusion injury in cardiac surgical patients.[4,5] Recently, with the development of newer filter materials for so called depth filters, specific filters for removing leukocytes have become clinical available. Depth filters promote the adhesion of leukocytes at the inside of the filter by mechanical and physical bonding and lead to a reduction of postoperative inflammatory response, especially for the lungs.[6,7]

A next logical step would be the removal of other specific substances such as lipids or cytokines by selective filtration. In this article we will review the composition of fat in the human body and the effects of fat particles generated during cardiac surgery and discuss the possible benefits of fat removal by filtration.

COMPOSITION OF FAT

Fat is a subset of the lipids. Fat is in fact an ester of fatty acids with glycerol, while the lipids form a large group of compounds that are insoluble in water and soluble in nonpolar solvents. Lipids in the human body can be distinguished in simple, compound and derived lipids.[8] The simple lipids are esters of fatty acids with glycerol as is shown in figure 1. These simple lipids form the majority of the body lipid stores as neutral triglycerides, i.e. without electrical charge. It should be noted however, that the triglycerides for the most part are mixed. This means that they consist of two or three different fatty acids. The major components of fatty acids in the body are palmitoleic acid, oleic acid, linoleic acid and arachidonic acid.

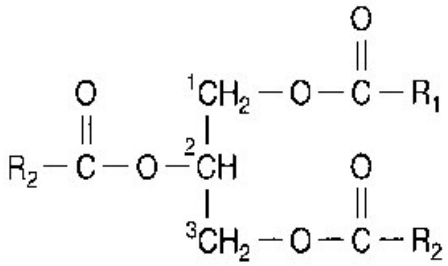

Figure 1. Triglyceride. This is the alcohol glycerol which has a fatty acid chain connected at each of its three binding sites, numbered 1-3. The acronyms R1 and R2 indicate that this triglyceride is composed of different fatty acids and is therefore a mixed triglyceride.

The compound lipids consist for most part of phospholipids and glycolipids. These compound lipids are mainly found in cellular membranes and in the myelinated parts of the central nervous system.

Derived lipids are derived both from the simple and compound lipids by hydrolysis and thus comprise fatty acids, glycerol, steroids and ketone bodies. Cholesterol belongs to the lipids. It is not in itself a fat as it is not an ester of an alcohol, but it is present in animal fat and is the basic compound of the steroids. It is predominantly synthetized by the body itself. Normal fat intake in a western adult is about 100 g triglyceride and 1 g cholesterol a day.

FAT RELEVANT TO CARDIAC SURGERY

The fat that we are interested in during cardiac surgery consists of the subcutaneous fat, fat around organs (for example around the heart and in the mediastinum), fat in the bone marrow and the fat that is present in the blood. The situation during cardiac surgery, however, is different from the situation during orthopaedic or trauma surgery, which are known for the generation of the fat embolism syndrome. Sternal bone contains red marrow which has about 5% fat, whereas the long bones yellow marrow which is present in contains about 85% fat.[9] Nonetheless, sternotomy has been associated with a rise in blood lipid content on the first postoperative day.[10]

Apart from the fat in the blood, we have to deal primarily with the neutral, simple lipids. Surprisingly few studies have the composition of the fat in the different body stores as a subject. It appears that the main fatty acid constituents of the subcutaneous fat are palmitic acid (19-21%), oleic acid (41-44%) and linoleic acid (10-12%).[11,12] Lipids in blood plasma are bound for about 75% to lipoproteins. These lipoproteins are water soluble complexes consisting of a layer of phospholipids covering triglycerides and cholesterol esters (figure 2).

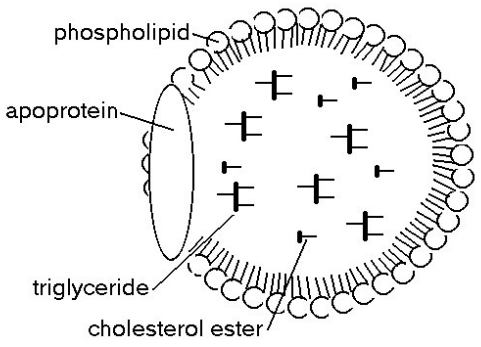

Figure 2. Lipoprotein particle. Cholesterol esters and triglycerides are located in the particle core. The apoprotein part reacts with cellular receptors.

The neutral fats can be broken down through the activity of the enzyme lipase A. This leads to the formation of glycerol and fatty acids. Thus during surgery we will encounter the intact neutral lipids and derived substances as glycerol, free fatty acids and ketone bodies. Although the quantity of free fatty acids is small, they are metabolic active. Depending on the number of unsaturated bindings they uncouple the oxidative phosphorylation and inhibit the ADT-ATP exchange across mitochondrial membranes.[13] This results in cell destruction.

ADVERSE EFFECTS OF FAT DURING CARDIAC SURGERY

Brain

Neurological damage after cardiopulmonary bypass (CPB) is a matter of major concern, responsible for increased postoperative morbidity.[14] The neurological damage can be linked to fat emboli through the studies of Moody and co-workers.[15] They investigated post-mortem brain slices from patients who died after cardiopulmonary bypass. Using specific staining for fat, they found many microemboli in the form of small capillary and arteriolar dilatations (SCAD). These fat emboli are thus associated with ischaemic brain injury.

Brooker and co-workers showed in a study with dogs, subjected to different forms of CPB that only the application of suction and retransfusion of wound blood was associated with an increase in density of SCADS.[16]

Lungs

In animal experiments, injection of free fatty acids, particular oleic acid, consistently produces the development of the acute respiratory distress syndrome.[17,18] It has also been shown that pulmonary epithelial permeability is increased after embolisation with oleic acid, but not after embolisation with neutral fat.[19] Microemboli consisting of fat have been shown by electron microscopy in lung tissue of patients after CPB.[20] Conversely, many patients with sepsis and acute respiratory distress after trauma surgery show fat substances in alveolar macrophages.[21]

Leucocytes

Oleic acid increases the activity of CD11b on leukocytes, thereby increasing the adherence properties on the vascular wall.[22] This could explain the neutrophil accumulation in the lungs and the development of respiratory distress syndrome.

Kidneys and other organ systems

Deposits of fatty material have also been demonstrated in kidney tissue at autopsy. However, this was after CPB using a bubble oxygenator.[23] More recently, lipid droplets could be demonstrated in urine samples from patients after cardiac surgery.[24]

PROPERTIES AND APPLICATION OF FAT REMOVAL FILTERS

At this moment a fat removal filter is available that consists of a polyester depth filter. This material is more or less the same as in leukocyte removal filters. However, the internal architecture is different, leading to effective lipid removal. Recently, in a

laboratory study with reconstituted outdated packed red blood cells, it was found that the filter removed 35% of the lipids.[25] The capacity of the filter is about 500 ml of blood, which may limit its use during cardiopulmonary bypass. As there is a large quantity of lipid microemboli in wound suction blood,[16,23] an application of a fat removal filter in the cardiotomy suction line would be a logical first step. Given a quantity of 1000ml for wound suction blood during CPB, this necessitates the use of two filters. Besides, an application in cardiotomy suction blood would place the filter in a low flow, low pressure circuit. Studies on leukocyte filtration have shown that the efficiency of the filter depends on the contact time between blood and the filter material.[26]

HOW TO MEASURE FAT

Fat can be measured as fat globules in blood. This gives an impression of the quantity of fat present and of the size of the emboli. After staining the fat globules can be counted and sized in a counting chamber. This old method is often used in conjunction with the clinical syndrome of fat embolism. Major drawback is that this method does not give qualitative data.

A more qualitative approach gives the thin layer chromatography.[27] On a coated glass plate a lipid mixture is applied. Then the plate is dipped in a solvent. Depending upon the affinity for the solvent, the different constituents are separated as is shown in figure 3. These separated fat components can be removed for further analysis.

Another approach is to measure directly the amount of triglycerides and cholesterol in the blood by biochemical assay. This measurement is specifically aimed at quantification of the neutral fat, which is biologically less active. The disadvantage of this method is thus that the metabolic active free fatty acids are not measured. Glycerol should in this setting also be measured. Glycerol is a necessary component of the triglycerides and when the triglycerides fall apart in free fatty acids the glycerol will be released. It is therefore also a measure for fat degeneration.

POSSIBLE CLINICAL APPLICATIONS

During CPB the blood removed from the wound by suction is commonly retransfused into the patient in order to reduce homologous blood transfusion requirements. The presence of a considerable quantity of microemboli in the wound suction blood was demonstrated by Solis and co-workers as early as 1974.[23] It would thus be beneficial for the patient to apply a fat removal filter for the cardiotomy suction blood. However, the capacity of the available filter is rather small for this application.

The filtration of the residual heart lung machine blood after cardiopulmonary bypass deserves investigation. This blood is infused in the patient and passes the lungs as a first organ. We have shown that leucocyte depletion of this blood improves postoperative oxygenation.[7] Fat filtration of this blood could have the same benificial effects. Furthermore, a major role for fat filtration should be in conjunction with the application of a cell-saver device. Blood processed by a cell-saver is known to contain a fat layer. The amount of fat present is dependent upon the type of cell-saver used.[28]

In conclusion fat filtration is a promising new application of the concept of filtering specific substances. At this moment investigations are under its way to determine the place of fat filtration in clinical practice.

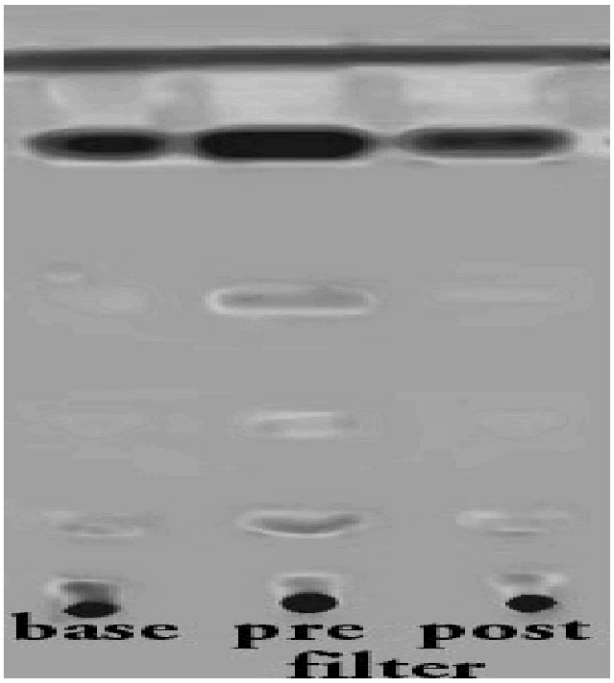

Figure 3. Thin layer chromatography showing the lipid profile in the plasma of a patient during cardiac surgery and the effects of wound suction and fat filtration on the different components of the lipid profile. The lipid profile shown on the left, indicated base, was taken just after induction of anaesthesia. The lipid profile shown in the middle, indicated pre, represents the wound suction blood. Triglycerides and free fatty acids are increased compared to base as indicated by a blacker and broader band. On the right, indicated post, the effect of lipid filtration on the wound suction blood is shown, indicating a reduction in triglycerides and free fatty acids compared to pre.

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CHAPTER 7

CLINICAL EVALUATION OF A NEW FAT REMOVAL FILTER DURING CARDIAC SURGERY.

A.J. de Vries, J. Gu MD, Y.L. Douglas, W.J. Post, H. Lip, W. van Oeveren

European Journal of Cardio-thoracic Surgery 25 (2004) 261-266

ABSTRACT

Fat microemboli are generated during cardiac surgery that are associated with postoperative organ injury. Recently, a fat removal filter has been developed, based on a polyester leucocyte depletion filter. However, the efficacy of such a filter in a clinical setting is unknown. In this study we tested the efficacy of this filter.

Coronary artery bypass patients were randomly divided in two groups. Group I: filtration of cardiotomy suction blood during cardiopulmonary bypass with a fat removal filter (n = 14). Group II: control patients without filtration (n = 14). Filter efficacy was evaluated in group I using biochemical assays and thin layer chromatography of blood samples taken simultaneously before and after the filter. In addition, clinical and biochemical markers for organ injury were determined in both groups.

We found that the fat filter removed triglycerides (0.9 ± 0.08 mmol.L^{-1} vs. 0.63 ± 0.08 mmol.L^{-1}, $p = 0.004$, paired *t*-test), leucocytes (4.3 ± 0.8 x 10^9 vs.2.3 ± 0.6 x 10^9 .L^{-1}, $p = 0.03$), and platelets (116 ± 26 x 10^9 vs. 75 ± 21 x 10^9 .L^{-1}, $p = 0.003$) from the blood samples taken before and after the filter. Chromatography showed a significant reduction in free fatty acids, phospholipids and triglycerides. Clinically, leucocyte counts were similar, but platelet counts were higher (181 ± 14 x 10^9.L^{-1} v. 117 ± 8.6 x 10^9.L^{-1} control, $p < 0.001$) in group I on the first postoperative day.

This study shows that the fat filter removed 40% fat, leucocytes and platelets from cardiotomy suction blood during cardiac surgery. A larger scale study is necessary to determine clinical effects on organ damage.

INTRODUCTION

Recently, fat microemboli have been demonstrated in brain tissue after cardiopulmonary bypass (CPB).[1] These were related to retransfusion of cardiotomy suction blood,[2] and associated with postoperative neurocognitive dysfunction.[3] Therefore, attention is again focussed on the adverse effects of retransfusion of cardiotomy suction blood during cardiac surgery. In addition, the role of fat on organ damage may have been underestimated, because fat microemboli have not only been demonstrated in brain tissue after CPB, but also in lung tissue.[4] In animal experiments, the injection of free fatty acids, particular oleic acid, consistently produces the development of an acute respiratory distress syndrome.[5] Finally, fat deposits have been demonstrated in kidney tissue[6] as well as in urine after CPB.[7]

Several strategies are used to prevent retransfusion of cardiotomy suction blood. Off-pump revascularization is increasingly performed, but is not suitable for intra-cardiac surgery. In some centers the cardiotomy suction blood is completely discarded,[8] but this may lead to increased allogenic transfusion requirements. Cell savers are used to wash the wound suction blood, but their use is expensive, and the quality of the processed blood is questioned.[9] Retransfusion of cardiotomy suction blood, however, is still used during CPB, and thus a novel approach with a simple and inexpensive filter for the removal of fat and debris from cardiotomy suction blood may be an alternative. Such a fat removal filter has been developed. This is a polyester 40 μm screen filter, which is based on a leucocyte removal filter and allows high flow transfusions. In a laboratory experiment this filter removed fat from reconstituted blood.[10] In patients however, little is known about the performance of a fat removal filter. The aim of this study was therefore to assess the efficacy of a fat removal filter in a clinical setting by filtering cardiotomy suction blood during CPB in patients undergoing coronary artery bypass grafting (CABG). As this study also served as a pilot study for cardiotomy blood filtration on postoperative organ injury, we evaluated some of the possible filter effects on lung, kidney and heart with clinical and biochemical markers, using an unfiltered group of CABG patients as controls.

MATERIAL AND METHODS

Patients

After institutional human investigation committee approval and patient consent 28 patients scheduled for CABG were prospectively randomized to Group I: fat filtration of cardiotomy suction blood during CPB (n = 14), or Group II: control patients without filtration (n = 14). Exclusion criteria were pre-existing lung disease, cerebral vascular disease, diabetes mellitus, emergency operation and re-operation. Blood samples were drawn from the radial artery (1) after induction of anaesthesia, (2) at the end of the operation, (3) after three hours in the ICU, and (4) on the morning of the first postoperative day. From blood samples taken pre-operatively and postoperatively on day 1, 2 and 6, the creatinin clearance was calculated according to the Cockcroft formulae.[11]

Anaesthesia and perfusion

Anaesthesia was induced and maintained by intravenous infusion of midazolam (0.1 $mg.kg^{-1}$) and sufentanil (1.5 $\mu g.kg^{-1}$), as previously described.[12] Pancuronium (0.1 $mg.kg^{-1}$) was used for muscle relaxation. Dexamethasone (1 $mg.kg^{-1}$) was given after induction. Ventilatory management was aimed at normocapnia throughout the operation and in the intensive care unit (ICU), with an inspiratory oxygen fraction of 0.4, a positive end-expiratory pressure of 6 cm H_2O and a tidal volume of 6-8 $mL.kg^{-1}$. Bovine lung heparin (300 $IU.kg^{-1}$) was used for anticoagulation. This was monitored by the celite activated clotting time (ACT) (International Technidyne, Edison, NJ, USA) and maintained at a value ≥ 400 s. After CPB, heparin was neutralized by protamine (300 $IU.kg^{-1}$).

The extracorporeal circuit consisted of roller pumps (Stöckert, München, Germany), a hollow fibre oxygenator (Sarns Turbo, 3M, St. Paul, MN, USA) and a standard arterial line filter (Affinity 38µ Medtronic, Minneapolis, MN, USA). The priming consisted of 500 mL hydroxyethylstarch 10% (Haes, Fresenius, Bad Homburg, Germany) and 1000 mL lactated Ringer's solution. Pump flow was adjusted to 2.4 $L.m^{-2}$ per min. Nasopharyngeal temperature during CPB was maintained at 32°C.

Filtration procedure

In the filter group the cardiotomy suction blood was collected in a separate cardiotomy reservoir (ATR120, Fresenius, Bad Homburg, Germany) from the moment that the ACT was ≥ 400 s. After aortic cross clamp release this cardiotomy blood passed under gravity through a fat removal filter (LipiGuard, Pall, Portsmouth, UK) into the cardiotomy reservoir of the CPB circuit. After each 600 mL of cardiotomy blood a new filter was used. In the control group the cardiotomy suction blood was collected directly in the cardiotomy reservoir of the CPB circuit from the moment that the ACT was ≥ 400 s.

In both groups, the residual blood in the extracorporeal circuit after CPB was collected in a transfusion bag and transfused into the patient using a standard transfusion system.

Measurements

When 200 mL blood had passed through the filter, samples were taken simultaneously before and after the filter. From EDTA-anticoagulated blood, haematocrit, platelet and total white blood cell counts were determined by an electronic cell counter (Cell-Dyn 610, Abbott, Santa Clara, CA, USA). Triglyceride levels were determined with a biochemical assay (Sigma, St. Louis, MO, USA).

To assess the capacity of the filters blood samples were taken from 4 additional filters in separate patients after 50 mL, 200 mL and 600 mL of blood had passed through the filter. From these samples platelet and total white blood cell counts, and triglyceride levels were measured as before.

In addition, to assess the qualitative effects of filtration, modified thin layer chromatography according Folch[13] was performed on samples before and after the filter and on a patient blood sample before CPB, as well as on the blood samples that were taken for the assessment of the filter capacity. Briefly, plasma was extracted with a chloroform-methanol mixture. The extract was mixed with butylated hydroxytoluene to avoid oxidation and after drying solved in chloroform. On a silica plate 10 µL

samples were applied. This was run with a mixture of n-hexane-diethylether-acetic acid and developed with cupper sulphate in fosforic acid. Five bands were discerned: cholesteryl esters, triglycerides, free fatty acids, cholesterol and phospholipids. For a semiquantitative evaluation of the chromatography, the bands were scanned by computer and the intensity of the bands was attributed a score from 0, being totally white to 100, being totally black. The values given in table 3 are these computerized density scores.

As we found a significant preservation of platelet counts after filtration including significant higher postoperative circulating platelet counts in the filtration group, we retrospectively analyzed the adsorption of the filter material of platelet activating factor. Therefore, we incubated 40 mg pieces of filter material with 0.05 mM purified platelet activating factor (PAF) C-18 (Sigma, St Louis, Mi, USA) which was then added to fresh platelet rich plasma from healthy volunteer blood. Platelet aggregation was compared between PAF C-18 with or without pre-incubation with filter material by means of optical aggregometry (Chronolog, Havertown, PA, USA).

Statistics

The sample size for this study was calculated on the assumption that the fat filter would remove at least 50% of the fat from the surgical cardiotomy suction blood. Twelve patients would therefore be needed with an power of 0.8 and an α of 0.05. All data are presented uncorrected for haemodilution and expressed as mean ± standard error unless otherwise stated. For comparison of single data between the groups a two-tailed Student's *t*-test was used. For comparison of the measurements before and after the filter a paired Student's *t*-test was used. Two way analysis of variance (ANOVA) for repeated measurements was used to determine the effects of time, group and interaction over the different time points. In case of multisample sphericity Greenhouse-Geisser (ϵ) adjustments were made. To allow for multiple comparisons the results were corrected using the least square difference method. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Both groups were similar with respect to age, sex, length, weight, haematocrit (p = 0.16), creatinin clearance, grafts and CPB time (p = 0.2). The demographic data are summarized in table 1.

Filter characteristics

The amount of cardiotomy suction blood was 1104 ± 152 mL. with a haematocrit of 19 ± 1.4%. Baseline plasma triglyceride level in the patients was 1.02 ± 0.15 mmol. L^{-1}. The filter removed 30% of the triglycerides and reduced leucocytes by 47% and platelets by 35% (table 2). Thin layer chromatography revealed that after filtration, free fatty acids (FFA), triglycerides and phospholipids were reduced (table 2). The efficacy of the filter decreased slightly during the 600 mL of blood that passed through the filter. After 600 mL of blood the filter removed 13% of the triglycerides and reduced leucocytes by 34% and platelets by 31%. Thin layer chromatography of

Table 1. Demographics

Group		Filter	Control
Age (yr)		62 ± 2.5	63 ± 3.2
Sex (male)		10	10
Length (cm)		176 ± 2.6	172 ± 2
Weight (kg)		81 ± 2.5	77 ± 2
Haematocrit (%)		33.5 ± 0.9	35.8 ± 1.3
Creatinin Cl ($mL.kg^{-1}$ per min)		76 ± 4	72 ± 7
CPB (min)		97 ± 9.2	83 ± 7.1
Grafts	arterial (n)	20	22
	venous (n)	20	19

Creatinin Cl, creatinin clearance according to the Cockroft formulae[11]; CPB, cardiopulmonary bypass.

Table 2. Filter performance

Samples	before the filter	after the filter.	*p*-level
Biochemical assays			
Triglycerides ($mmol.L^{-1}$)	0.9 ± 0.09	0.63 ± 0.07	0.003
Leucocytes ($x10^9.L^{-1}$)	4.3 ± 0.8	2.3 ± 0.6	0.03
Platelets ($x10^9.L^{-1}$)	116 ± 26	75 ± 21	0.003
Chromatography			
Free fatty acids	7.6 ± 1.1	4.1 ± 0.8	0.005
Triglycerides	6.4 ± 1.0	3.4 ± 1.1	0.01
Phospholipids	49.3 ± 3.4	45.3 ± 3.8	0.04
Cholesteryl esters	35.1 ± 2.8	31.9 ± 2.7	0.11
Cholesterol	9.8 ± 1.4	9.7 ± 1.7	0.91

Values given for the chromatography are the computerized density scores. See text for explanation. The *p*-values reflect the statistical analysis of the samples taken before and after the filter by one-way Student *t*-test.

Table 3. Clinical results on the first postoperative day and hospital stay

Group	Filter	Control	*p*-level
Cr Cl ($mL.kg^{-1}$ per min	101 ± 6.5	79 ± 7.9	0.04
Fluid in (mL)	4040 ± 262	4072 ± 291	0.94
Blood loss (mL)	928 ± 126	753 ± 99	0.29
Diuresis (mL)	2920 ± 215	3183 ± 308	0.49
CK-enzymes ($IU.L^{-1}$)	236 ± 56	169 ± 27	0.32
CK-MB ($IU.L^{-1}$)	12 ± 6.4	6 ± 1.8	0.44
Platelets ($x10^9.L^{-1}$)	181 ± 14	117 ± 9	
	<0.001		
Leucocytes ($x10^9.L^{-1}$)	15.6 ± 0.9	13.5 ± 1	0.13
Hospital stay (day)	7.2 ± 0.8	10.8 ± 1.5	0.05

Cr Cl, renal creatinin clearance according to the Cockroft formula[11]; CK, creatinin kinase; MB, myocardial band creatinin iso-enzyme.

these four filters after 600 mL revealed the same pattern as after 200 mL, with reductions in FFA, triglycerides and phospholipids. The time needed to pass 200 mL of blood under gravity at a height of 90 cm was 2 min 40s ± 13s, and for 600 mL this time was 7 min ± 20s. We found no adsorption of (hydrophobic) platelet activating factor (PAF) on the filter material, excluding its effect on the preservation of the blood platelets.

Clinical effects

The calculated creatinin clearance was higher in the filter group on the first postoperative day ($p = 0.04$) (tables 1 and 3). The two groups were similar with respect to fluid intake, diuresis, blood loss, lung function and myocardial injury (table 3). In the control group, one patient had a myocardial infarction (defined as new Q-wave on the ECG and CK > 180 U/L with CK-MB > 10% of total), one patient had major blood loss and one patient developed renal function disturbances with a serum creatinin level of 231 mmol.L^{-1}. Overall hospital stay was slightly shorter in the filter group (table 3). It is noted that the attending ICU and hospital staff were blinded to the study groups.

The PaO_2 showed a time effect ($p = 0.001$), but there was no difference between the groups ($p = 0.25$) (figure 1). The A-a gradients showed a time effect ($p < 0.001$), but no difference between the groups ($p = 0.25$) (figure 1).

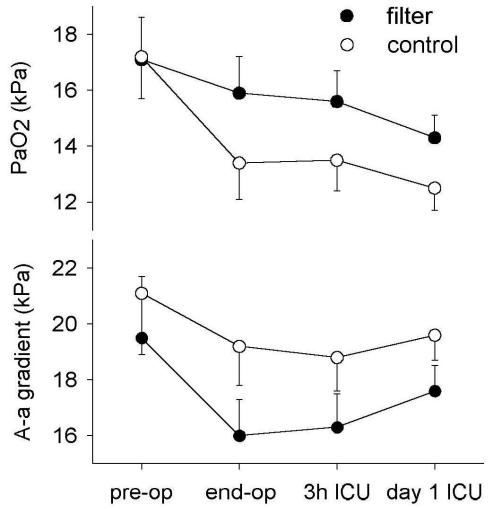

Figure 1. Arterial oxygen tension (PaO_2) and alveolar-arterial (A-a) oxygen gradients in the fat filter group and in the unfiltered control group preoperatively (pre-op), at the end of operation (end-op), after 3 hours in the intensive care unit (3h ICU) and on the morning of the first postoperative day (day 1). Values shown are the means, estimated by the repeated measurement model with standard error. The PaO_2 showed a time effect ($p = 0.009$), but no difference between the groups. The A-a gradients showed a time effect ($p < 0.001$), but no difference between the groups.

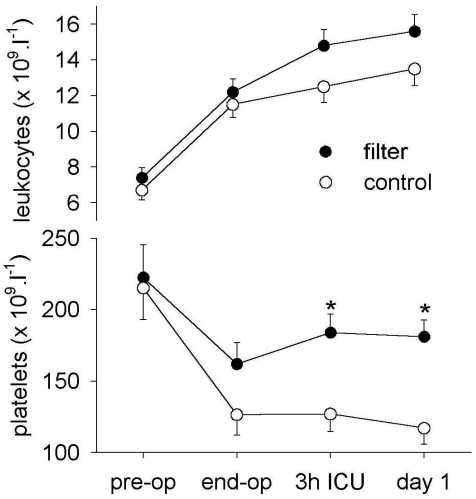

Figure 2. Circulating leucocyte and platelet counts in the filter group and in the unfiltered control group preoperatively (pre-op), at the end of operation (end-op), after 3 hours in the intensive care unit (3h ICU) and on the morning of the first postoperative day (day 1). Values shown are the means, estimated by the repeated measurement model with standard error. Circulating leucocyte counts showed a significant time effect ($p < 0.001$) by repeated measurements analysis of variance. The circulating platelet counts were different ($p < 0.05$). Asterisk indicates significant ($p < 0.05$) differences at separate time points by Student's *t*-test.

The postoperative platelet counts on the first postoperative day were higher in the filter group than in the control group (figure 2, table 3). There was a time effect ($p < 0.001$) and a difference between the groups ($p = 0.04$). The postoperative circulating leucocyte counts were similar in both groups. There was a time effect ($p < 0.001$), but there was no difference between the groups ($p = 0.08$) (figure 2).

DISCUSSION

This study showed that the application of a fat removal filter reduced the fat content of cardiotomy suction blood in cardiac surgical patients. The filter removed 46% of the free fatty acids and 30% of the triglycerides as shown by thin layer chromatography and plasma samples.

The mechanism for fat removal is not clear. The filter consists of tightly packed fibers with a porous structure of about 40 μm. This may mechanically stop the larger fat globules. Such a view is supported by a recent study on cardiotomy suction blood.[14] Fat microemboli were divided in large (> 50 μm) and small (10-50 μm) size emboli. In a subset of 6 patients an additional filter was placed after the cardiotomy reservoir. No large emboli were detected after the filter. In our filter the removal of the various fat subgroups was highly variable. This may be explained by a difference in electrostatic

adhesion to the filter material. The thin layer chromatography supports this view, because the more polarized substances as the free fatty acids were removed more effectively. One could therefore speculate on filter improvement by coating of the fibres to increase the removal of the other subgroups, but clinically the free fatty acids appear to be the most important. Increased levels of free fatty acids have documented effects. In pancreatic tissue β cells are damaged.[15] In kidney tissue tubulointerstitial damage is aggravated.[16] In lung tissue free fatty acids are associated with the development of an acute respiratory distress syndrome.[5] In endothelial cells free fatty acids cause vasoconstriction and granulocytes are activated through surface expression and activity of CD11b.[17]

We found a lower overall efficacy of the filter in the clinical setting of our study than previously reported in a laboratory setting with reconstituted blood.[10] It has recently been shown that the composition of the cardiotomy suction blood is different, and that a low temperature increases filter efficacy.[18] This could explain our results and is supported by another clinical study that also showed a moderate efficacy of this filter in 3 orthopaedic patients.[19] Free fatty acids are bound to albumin. Plasma albumin is reduced by haemodilution after CPB. For this reason we did not use a prime with albumin, but instead used hydroxyethylstarch, which is not known to interfere with binding of free fatty acids.

With about 85 mL/min the filter appeared to have a high flow during transfusion under gravity. However, a high flow reduces the contact time between blood and filter medium and thus may result in a lower filter efficiency.[20] Thus, filter efficiency may be improved by coating the fibers, or alternatively by packing more filter materials in the housing. This latter option would reduce the flow over the filter. However, a flow of 30 mL/min should be sufficient to filter 1.5 L, which equals the amount of cardiotomy suction blood, during a cross clamp time of 45 min. For widespread use the fat removal filter will need a larger capacity, as our results indicated that the filter became saturated after 600 mL, requiring to change it.

We did not measure lipoprotein levels in this study. Lipoproteins consist of a layer of phospholipids which covers triglycerides and cholesterol esters. These complexes are necessary to facilitate lipid transport through the plasma compartment. The objective of the identification of the several subgroups of lipoproteins lies in their contribution to the atherosclerotic risk profile. That was not the purpose of this study. Moreover, we speculated that fat release during the operation would mainly result from mechanical damage through surgical manipulation and shear forces. This would result in a direct release of the triglycerides and free fatty acids, which we measured.

Several clinical findings in this small pilot study suggest a beneficial effect of the filter. First, the higher calculated creatinin clearance in the filter group on the first postoperative day in view of a similar postoperative fluid balance. Fat emboli have been demonstrated in the kidney after CPB,[6] and also after experimental fat embolism syndrome.[21]

The second is the higher postoperative platelet counts in the filter group. Platelets and leucocytes in the cardiotomy suction blood are activated in the presence of fat and tissue factor from the pericardium.[22] Thus, removal of platelets and leucocytes by the filter may be advantageous and protective against the systemic inflammatory response and thrombus formation.

It has been reported that activated platelets do not remain in the circulation but are actively cleared.[23] This may explain the higher postoperative circulating platelet counts in the filter group, suggesting that the platelets were less activated than in the control group. Direct adsorption of platelet activating factor by the filter was not shown as a mechanism of higher circulating platelet counts after filtration. We have not determined β-thromboglobulin levels, as the effects of the filter on the circulating platelet counts were not expected. Measurement of leucocyte activation, for example by determination of CD11/CD18, could have clarified the slightly higher postoperative circulating leucocyte counts in the filter group, because it is known that free fatty acids result in surface expression and activity of CD11b on human neutrophils.[17]

Third is postoperative oxygenation. Although not significant different in itself due to the small sample size, the fact that the postoperative A-a gradients were smaller, and the postoperative PaO_2 values were higher in the filter group suggest that in the filter group less pulmonary injury occurred. This may be explained by the fact that the filter significantly reduced free fatty acids, known for their deleterious effects on lung function.[5] In addition, the filter also removed a significant part of the leucocytes from the suction blood. We have previously shown that filtration of leucocytes improved postoperative lung function.[24]

Several other possibilities for the management of the cardiotomy suction blood exist. Cell savers are increasingly used, but these devices might be less than ideal for several reasons. First, fat is not completely removed by cell savers.[25,26] Thus, as a consequence, even cell saver blood may benefit from the application of a fat removal filter before retransfusion. Second, their use is expensive and requires attention and time to process. In contrast, fat removal filters are cheaper, about 25% of the cost of a cell saver, they are very easy to operate and processed blood is immediately available. Kaza found cell savers not more effective than the application of a filter after the cardiotomy reservoir for the elimination of small and large fat emboli.[14] Third, processed cell saver blood contains increased levels of interleukin-1[9] and activated leucocytes,[27] which may aggravate the inflammatory reaction associated with CPB.

There are shortcomings in this study. It was underpowered to detect clinical differences between the groups. Based on our results, at least 35 patients in each group had to be included to demonstrate clinical differences with a power of 0.8 and an α of 0.05. However, our results suggest that it would be worth to perform such a study. Further, we use routinely dexamethasone for all our patients to reduce the inflammatory reaction after CPB. The incidence of the fat embolism syndrome was decreased in a prospective randomized clinical trial, where steroids were given to prevent the effects of the fat embolism syndrome.[28] Therefore, the effects of the fat removal filter on organ damage could be more pronounced than demonstrated in this study. Third, we did not use a separate cardiotomy reservoir in the control group. Instead, the cardiotomy blood was gradually mixed with the patients' blood during the whole CPB period as usual. This gradual mixing may have reduced the effects of the transfusion of cardiotomy blood in the control group.

In conclusion, our results demonstrate that the fat removal filter removed approximately 40% of fat, leucocytes and platelets from cardiotomy suction blood. The efficiency and capacity of the filter should be improved and a prospective study of the effects on postoperative organ damage should be performed. The application of a fat filter however, is not the ultimate answer to a reduction of microemboli. It is estimated

that 60% of the emboli during surgery are caused by surgical manipulation.[3] However, the presence of cerebral fat microemboli justify that every effort is done to reduce the fat load for the patient.

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CHAPTER 8

THE CLINICAL APPLICATION OF A FAT REMOVAL FILTER: BIOCHEMICAL RESULTS AND CELL COUNTS.

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Submitted

ABSTRACT

Retransfusion of cardiotomy suction blood during cardiac surgery is associated with cerebral microemboli and an inflammatory reaction. Recently, a fat removal filter has been introduced, but little is known about the clinical effects. To assess the effects of this filter we measured biochemical markers and cell counts in patients.

Randomized prospective study in elective cardiac surgical patients (n = 28). During cardiopulmonary bypass the cardiotomy suction blood was filtered with a fat removal filter and retransfused in 14 patients (filter). In 14 patients the cardiotomy suction blood was discarded (waste). Triglyceride and glycerol, and neuron-specific enolase (NSE) and S-100β as brain injury markers, and circulating total white blood cell and granulocyte counts and interleukin-6 as inflammatory markers were measured.

The filter removed triglycerides (0.9 ± 0.08 mmol.L^{-1} v. 0.63 ± 0.08 mmol.L^{-1}, p = 0.004), leucocytes (4.3 ± 0.8 x 10^9.L^{-1} v. 2.3 ± 0.6 x 10^9.L^{-1}, p = 0.03) and platelets (116 ± 26 x 10^9.L^{-1} v. 75 ± 21 x 10^9.L^{-1}, p = 0.003, paired t-test) from the cardiotomy blood. Apart from a transient increase in S-100β and NSE values in the filter group, there was no difference between the groups on the first postoperative day (S-100β 0.28 ± 0.05 μg.L^{-1} filter vs. 0.29 ± 0.04 μg.L^{-1} waste; NSE 16.7 ± 1.5 ng.L^{-1} filter vs. 14.9 ± 0.9 ng.L^{-1} waste). Triglyceride levels on the first postoperative day were similar. Total white blood cell (p = 0.009) and granulocyte counts (p = 0.01, both repeated measurements ANOVA) were higher in the filter group.

The filtration related transient increase in brain markers and the higher white blood cell and granulocyte counts in the filter group suggest that the filter efficacy should be improved.

INTRODUCTION

Retransfusion of cardiotomy suction blood during cardiopulmonary bypass (CPB) is used in cardiac surgery as a cost effective way to reduce the number of allogenic blood transfusions.[1] However, this practice may be questioned for at least two reasons. In the first place, cardiotomy suction blood that is retransfused, contains many fat emboli.[2] The use of cardiotomy suction is associated with an increase in cerebral emboli.[3] These emboli are largely responsible for the postoperative neurocognitive dysfunction that affects up to 30% of the patients 3 months after cardiac surgery.[4,5]

A second problem with retransfusion of wound blood is an inflammatory reaction in the patient. The activation of cardiotomy blood in the presence of fat and tissue factor from the pericardium [6] leads to a high concentration of platelet- and leucocyte-derived microparticles which are involved in the systemic inflammatory response after CPB.[7,8] Increased concentrations of the pro-inflammatory agent interleukin (IL)-6 in wound blood have also been related to febrile reactions after retransfusion.[9]

Recently, a fat removal filter has been developed that is suitable for retransfusion of wound blood. This is a high flow polyester screen filter, based on a leucocyte removal filter. During cardiac surgery a beneficial effect from the application of this filter was suggested [10], and a moderate clinical effect was observed in orthopaedic surgery.[11] For this study we hypothesized that the application of a fat removal filter for the cardiotomy suction blood would have a positive effect on brain injury and the inflammatory response after CPB as assessed by biochemical markers and cell counts.

Serum levels of the brain injury markers neuron specific enolase (NSE) and S-100β can be measured, but S-100β has been demonstrated in cardiotomy suction blood.[12] However, NSE and S-100β can still be used as markers for brain dysfunction if extracerebral sources are controlled for.[13] The best option for control is to discard the cardiotomy suction blood completely. This has the additional advantage that the systemic inflammatory response after CPB also will be minimized. Therefore, we compared in this study a group of patients in which we filtered and retransfused the cardiotomy suction blood during CPB, with a control group of patients in which we discarded the cardiotomy suction blood completely. Use of an effective fat removal filter would result in similar postoperative values for markers of brain injury and inflammation in both groups. As markers for brain injury we measured the serum levels of NSE and S-100β, triglycerides and glycerol, and as inflammatory markers total white blood cell, granulocyte and platelet counts and IL-6 levels.

MATERIAL AND METHODS

Patients

After institutional human investigation committee approval and patient consent 28 consecutive patients scheduled for elective coronary artery bypass grafting were prospectively studied. They were on the operating day with a computer generated randomization table allocated to either a filter group (n = 14), in which cardiotomy suction blood throughout the CPB period was filtered and retransfused, or a waste group (n = 14), in which the cardiotomy suction blood throughout the operation was discarded. This number of patients was calculated as follows. We estimated that a

difference of one standard deviation between the S-100β and NSE values after induction of anaesthesia and on the morning of the first postoperative day would be clinically relevant. It was therefore estimated that 14 patients in each group would be required to have a power of 0.8 at an α of 0.05 in order to detect a significant difference among the groups. Patients with redo-operations, with pre-existing cerebral disease or with a recent (<1 month) myocardial infarction were excluded.

Anaesthesia and perfusion

Anaesthesia was induced and maintained according to an established protocol[14] and consisted of infusion of midazolam (0.1 $mg.kg^{-1}$) and sufentanil (1.5 $\mu g.kg^{-1}$). Bovine lung heparin (300 $IU.kg^{-1}$) was used for anticoagulation. This was monitored by the celite activated clotting time (ACT)(International Technidyne Co., Edison, N.J., USA) and maintained at a value ≥ 400 s. After CPB, heparin was neutralized by protamine (300 $IU.kg^{-1}$). The extracorporeal circuit consisted of roller pumps (Stöckert Istrumente, München, Germany), a hollow fibre oxygenator (Sarns Turbo, 3M, St. Paul, Minn., USA) and an arterial line filter (Affinity 38μ, Medtronic, Minneapolis, Minn., USA). The priming consisted of 500 mL hydroxyethylstarch 10% (Haes 10%, Fresenius, Bad Homburg, Germany) and 1000 mL lactated Ringer's solution. Pump flow was adjusted to 2.4 $l.m^{-2}.min^{-1}$. Nasopharyngeal temperature during CPB was maintained at 32°C and α-stat pH-management was used.

Filtration procedure

In the filter group, the cardiotomy suction blood was collected in a separate cardiotomy reservoir (ATR120, Fresenius, Bad Homburg, Germany) from the moment that the ACT was ≥ 400 s. After aortic cross clamp release this wound suction blood passed under gravity through a fat removal filter (LipiGuard, Pall Biomedical, Portsmouth, GB) into the cardiotomy reservoir of the CPB circuit. After each 600 mL of suction blood a new filter was used in order not to exceed the recommended filter capacity.

In the waste group, the cardiotomy suction blood was aspirated with the hospital wall suction system and discarded.[15]

After CPB, the residual blood in the heart-lung machine was collected in a transfusion bag and in both groups retransfused to the patients. Postoperative shed mediastinal blood was not retransfused. Postoperative transfusion of homologous blood products was according to our hospital guidelines. The staff of the intensive care unit (ICU) was blinded to the study groups.

Measurements

For all laboratory tests and biochemical assays EDTA and citrate anticoagulated blood was drawn from the patients' radial artery catheter. Blood samples were drawn (1) after induction of anaesthesia, before the start of CPB, (2) at the end of the operation, (3) after three hours in the ICU and (4) on the morning of the first postoperative day. For biochemical assays, plasma was obtained by centrifugation of whole bood at 1000g and immediately stored at –80°C for further determinations. Serum levels of S-100β and neuron specific enolase (NSE) were both determined using enzyme immunoassays (Sangtec Medical, Bromma, Sweden). Interleukin-6 was determined using an enzyme immunoassay (Quantikine, R&D Systems, Minneapolis,

Minn, USA). Plasma levels of glycerol and triglyceride were both determined by routine biochemical methods (Sigma. St. Louis, MO., USA). Haemoglobin, haematocrit and platelet, total white blood cell and granulocyte counts were determined by an electronic cell counter (Cell-Dyn 610, Abbott, Santa Clara, CA, USA). Levels of triglycerides, and leucocyte and platelet counts were measured in addition from EDTA and citrate anticoagulated samples taken simultaneouly before and after the filter to assess the efficacy of the fat removal filter.

Statistics

All data are presented uncorrected for haemodilution and expressed as mean ± standard error. For comparison of single data between the groups a two tailed Student's *t*-test was used. For comparison of the measurements before and after the filter the paired Student's *t*-test was used. To identify group, time, and group-time interactions two way analysis of variance (ANOVA) for repeated measurements was used. To allow for multiple comparisons the Bonferroni adjustment was applied. Correlations were calculated by means of the Pearson correlation coefficient. A *p*-value ≤ 0.05 was considered statistically significant.

RESULTS

All patients that were included completed the study. The demographic data are summarized in table 1, which shows that both groups were similar. The postoperative clinical data are summarized in table 2, and indicate that there were no differences between both groups.

There were no complications requiring a prolonged hospital stay. The fat filter removed triglycerides (0.9 ± 0.08 mmol.L^{-1} v. 0.63 ± 0.08 mmol.L^{-1}, $p = 0.004$), glycerol (5.7 ± 0.37 mmol.L^{-1} v. 4.5 ± 0.44 mmol.L^{-1}, $p = 0.05$), leucocytes (4.3 ± 0.8 x 10^9.L^{-1} v. 2.3 ± 0.6 x 10^9.L^{-1}, $p = 0.03$) and platelets (116 ± 26 x 10^9.L^{-1} v. 75 ± 21 x 10^9.L^{-1}, $p = 0.003$) from the cardiotomy suction blood (1103 ± 154 mL). Compared to the baseline preoperative serum levels in the patients (figures 1 and 2), leucocyte and platelet counts were lower in the cardiotomy suction blood, the triglyceride levels were similar, but the glycerol levels were higher.

The release pattern over the time of the brain marker S-100β was different in both groups. A significant interaction between group and time was observed ($p = 0.006$, figure 1). This was due to a transient peak at the end of the operation in the filter group. On the first postoperative day however, S-100β serum levels were similar again (0.28 ± 0.05 μg.L^{-1} filter vs. 0.29 ± 0.04 μg.L^{-1} waste, figure 1). The NSE serum levels also showed a transient peak in the filter group, but there was no significant interaction between group and time ($p = 0.07$, figure 1). This peak was about 3 hours postoperatively. However, repeated measurements ANOVA revealed that the two groups were not different ($p = 0.37$, figure 1). The NSE serum levels in both groups on the first postoperative day were higher than the preoperative serum levels (figure 1), but this was not the case for the S-100β levels. The NSE serum levels at the end of the operation showed a weak positive correlation with the S-100β serum levels at the end of the operation ($r = 0.4$, $p = 0.04$).

Table 1. Demographics

Group	Filter (n = 14)	Waste (n = 14)
Age (yr)	62 ± 2.5	67 ± 2.2
Male (n)	10	10
Height (cm)	176 ± 3	175 ± 2
Weight (kg)	81 ± 2	82 ± 4
Hypertension (n)	3	7
Previous myocardial infarction (n)	3	3

Table 2. Clinical data

Group	Filter (n = 14)	Waste (n = 14)	p-value
CPB (min)	97 ± 9	91 ± 6	0.59
Intubation time (hours)	16.9 ± 0.9	17.5 ± 0.9	0.62
ICU stay (hours)	21.1 ± 0.5	21.8 ± 1.1	0.56
Hospital stay (day)	6.8 ± 0.5	6.8 ± 0.3	0.33
Chest tube drain ICU (mL)	928 ± 126	742 ± 120	0.30
Packed cell transfusion (mL)	243 ± 85	275 ± 136	0.85
Haemoglobin day1 (mmol. l^{-1})	6.2 ± 0.2	6.2 ± 0.2	0.85

Analysis of the glycerol levels over the time revealed a difference between the two groups (p = 0.04, figure 1), but this was not the case for the triglyceride levels (p = 0.49, figure 1). The glycerol and triglyceride serum levels on the first postoperative day were not different between the groups (triglycerides 0.63 ± 0.07 mmol.L^{-1} filter vs. 0.73 ± 0.11 mmol.L^{-1} waste; glycerol 1.27 ± 0.18 mmol.L^{-1} filter vs. 1,11 ± 0.2 mmol.L^{-1} waste, figure 1).

The total white blood cell counts increased during the study period ($p < 0.001$) and were different between the groups (p = 0.009, figure 2). This resulted in higher total white blood cell counts in the filter group on the first postoperative day. Similarly, the granulocyte counts, as the more reactive part of the white blood cells, increased during the study period ($p < 0.001$) and were higher in the filter group (p = 0.01, figure 2). A positive correlation between the granulocyte counts and the CPB time was present in the waste group (r = 0.69, p = 0.007), but this was not the case in the filter group (r = 0.21, p = 0.48).

Although the IL-6 levels were higher in the waste group on the first postoperative day (33.6 ± 5.9 ng.L^{-1}) than in the filter group (15.0 ± 5.7 ng.L^{-1}), there was no difference between the two groups over the time (p = 0.43, figure 2).

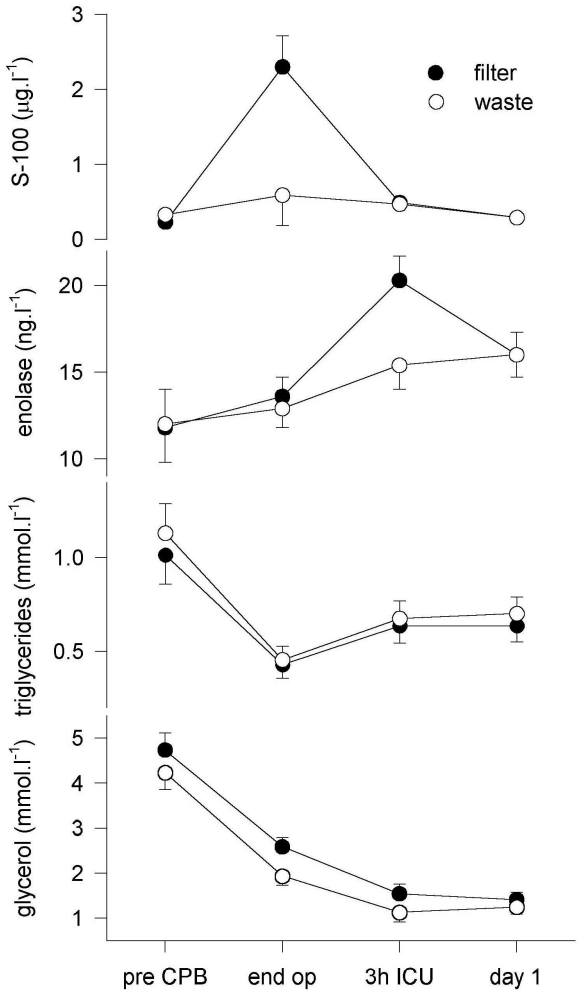

Figure 1. Serum S-100β, neuron-specific enolase, triglyceride and glycerol values in a group of patients where the wound suction blood was filtered and retrans-fused (filter) and a group of patients where the wound suction blood was discarded (waste), measured before cardiopulmonary bypass (pre CPB), at the end of the operation (end op), after 3 hrs in the intensive care unit (3h-ICU) and on the first postoperative day (day 1). Analysis of variance for repeated measurements indicates a difference between the groups for the S-100β βvalues ($p = 0.006$) and for the glycerol values ($p = 0.04$)

DISCUSSION

In this study in cardiac surgical patients, we did not observe different serum levels of NSE and S-100β on the first postoperative day, whether we retransfused the cardiotomy suction blood after passage through a fat removal filter, or completely discarded this blood. This finding suggests that the application of a fat removal filter is effective. However, we also observed a transient increase in serum levels of NSE and S-100β in the filter group. The serum levels of S-100β had a peak at the end of the operation, whereas the serum levels of NSE had a peak about 3 hours later. In addition, the glycerol levels and the total white blood cell and granulocyte counts, as inflammatory markers, were higher in the filter group. These findings suggest that the efficacy of the filter should be improved.

NSE and S-100β are both sensitive and early markers for brain injury.[16,17] NSE levels increase by cell destruction in the gray matter. An increase in serum NSE on the first postoperative day is associated with neuropsychological dysfunction.[18] In contrast, S-100β is released from cells in the white matter. Increased S-100β levels on the first

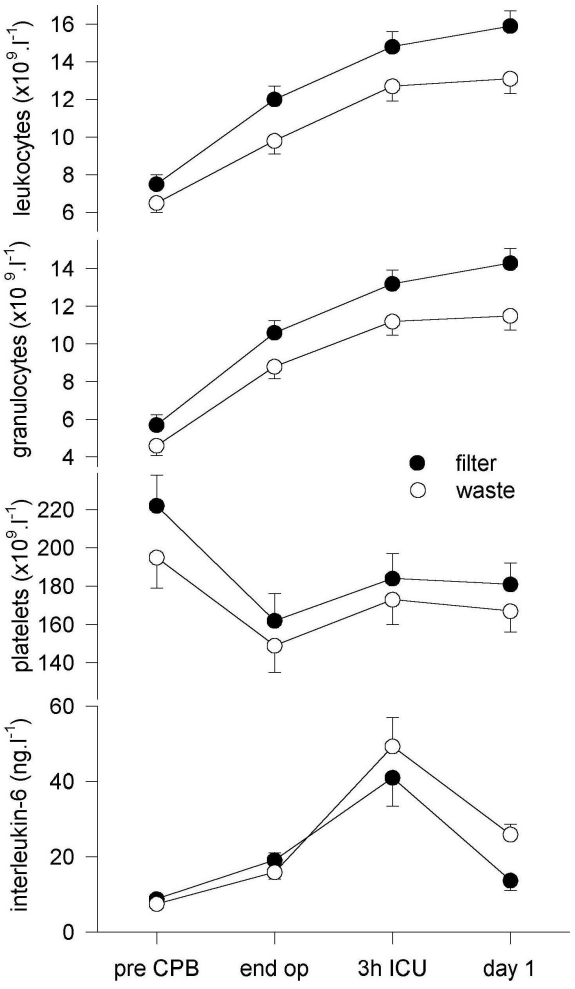

Figure 2. Total white blood cells (WBC), granulocytes, platelet counts and interleukin-6 levels in a group of patients where the wound suction blood was filtered and retransfused (filter) and a group of patients where the wound suction blood was discarded (waste), measured before cardiopulmonary bypass (pre CPB), at the end of the operation (end op), after 3 hours in the intensive care unit (3h-ICU) and on the first postoperative day (day 1). Analysis of variance for repeated measurements indicates a difference between the groups for WBC ($p = 0.009$) and granulocytes ($p = 0.01$)

postoperative day are associated with cerebral injury, but may be difficult to evaluate after cardiac surgery due to extracerebral S-100β. sources.[12] Thus, depending on the type of cell damage, the combination of the NSE and S-100β. release may be more specific for brain injury,[16,19,20] and we determined therefore both NSE and S-100β serum levels.

The clinical efficacy of the fat removal filter appeared insufficient for several reasons, despite the significant reduction in triglycerides, glycerol, leucocytes and platelets in the retransfused cardiotomy blood, and despite the similar NSE and S-100β serum levels on the first postoperative day in both groups. In the first place, the postoperative increase in NSE suggests at least some brain injury in the filter group, because it has been shown that NSE did not increase after abdominal surgery.[21] A second argument for an insufficient clinical efficacy of the fat filter, is the higher total white blood cell and granulocyte counts in the filter group. Moreover, in the waste group the postoperative total white blood cell and granulocyte counts correlated with the CPB time as was expected.[22] This was not the case in the filter group, and suggests that retransfusion of the filtered cardiotomy suction blood had a more profound effect on the inflammatory parameters than CPB itself.

A low filter capacity might explain the insufficient filter efficacy, but this is less likely as we frequently changed the filter according to the manufacturer's instructions. Others however, also felt that the filter efficacy should be improved. After orthopaedic surgery, postoperative wound blood was passed through a fat reducing filter which was found to be inferior to a leucocyte depletion filter.[11] This finding is supported by a laboratory study with reconstituted blood and soya oil, in which a leucocyte depletion filter was also more effective than the fat removal filter.[23]

We confined ourselves to the assessment of biochemical markers because of the small scale of this study. We also did not estimate the number of fat microemboli, but instead used triglyceride and glycerol measurements for several reasons. It has already been demonstrated that the application of a filter before retransfusion of cardiotomy blood prvented the passage of fat emboli larger than 50 μm.[24] This is most likely based on the mechanical removal of the fat emboli. Moreover, the assessment of the number and size of the microemboli reflects only neutral fat, which is biologically not active. During surgery however, triglycerides break down in glycerol and free fatty acids. Especially the free fatty acids are associated with organ damage, for example in the lungs.[25] These slightly polarised substances are also removed by the filter through the electrical charge of the fibers.[10] Therefore the glycerol and triglyceride measurements may more accurately reflect organ damage.

The neurological effects of clinically applied filtration techniques have not been assessed before. Only Kincaid et al. processed cardiotomy suction blood in dogs with a cell saver and passed this blood through a leucocyte depleting filter in a subset of 6 dogs. They found no difference in cerebral microemboli compared to processed, but unfiltered blood.[26] Their findings support our results with respect to the NSE and S-100β serum levels on the first postoperative day. However, serum levels of brain injury markers may not directly be related to clinical impairment, as a deficit in one region of the brain may have more pronounced clinical effects than that same deficit in another region of the brain. Consequently, a larger scale study is necessary to demonstrate clinical important differences.

In conclusion, the results of this study do not demonstrate a difference in S-100β and NSE values on the first postoperative day. However, the filtration related transient increase in brain injury markers and the higher leucocyte and granulocyte counts suggest that the filter efficacy should be improved. A larger scale clinical study is therefore necessary before widespread application of a fat removal filter can be recommended.

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CHAPTER 9

SUMMARY AND CONCLUSIONS

Activated leucocytes play a key role in the generalized inflammatory response that occurs after cardiac surgery. They have a pivotal role in reperfusion injury after ischaemia, and also interact with the vascular endothelium and the cardiopulmonary bypass circuit. This generalized inflammatory response leads to postoperative tissue injury. To minimize or even prevent postoperative tissue injury, it is thus indicated to modify the effects of leucocytes. Various anti-inflammatory strategies have been tried, such as the coating of the cardiopulmonary bypass circuit, pharmacological interventions as the use of corticosteroids and aprotinin and the use of a cell-saver for the washing of wound blood. Removal of leucocytes by means of a filter, however, seems to be the most effective treatment. Good results have been achieved with leucocyte depletion in the setting of ischaemia and reperfusion. However, not only activated leucocytes are associated with postoperative tissue injury, but also retransfused fat. Fat emboli have been demonstrated in the brain after cardiac surgery and are associated with ischaemic brain injury. Fat emboli have also been demonstrated in lung and kidney tissue.

Improved filter technology may thus include the removal of various harmful substances such as fat from the blood. Thus, the aim of this thesis as outlined in chapter 1, was to demonstrate that leucocyte and fat filtration, applied in the setting of cardiac surgery, have a beneficial effect on inflammatory markers and postoperative organ injury.

Conflicting results have been reported about the clinical effects of leucocyte depletion with an arterial line filter during the whole period of cardiopulmonary bypass. Therefore, in chapter 2 a new leucocyte depletion method is described. In a randomized prospective study in thirty patients undergoing elective cardiac surgery, we investigated whether leucocyte depletion from the residual heart-lung machine blood at the end of cardiopulmonary bypass would improve lung function and reduce the postoperative inflammatory response. In the leucocyte-depletion group all residual blood was filtered by leucocyte depletion filters before reinfusion in the patient, whereas in the control group an identical amount of residual blood was reinfused without filtration. In the leucocyte-depletion group, circulating leucocytes and granulocytes were reduced, and the postoperative arterial oxygen tension was higher one hour after arrival to the intensive care unit and after extubation. These results suggest that leucocyte depletion of the residual heart-lung machine blood improves postoperative lung gas exchange function and reduces the inflammatory response.

In chapter 3, a similar study is described in children presenting for congenital heart surgery. The inflammatory response after cardiopulmonary bypass in children is more severe than in adults. In addition, cyanotic children are also more vulnerable to oxygen radicals. We therefore expected a better clinical effect of leucocyte depletion of the residual heart-lung machine blood in children than in adults. The residual heart-lung machine blood was filtered with a leucocyte depletion filter in 25 children before reinfusion. A control group of 25 children received this blood unfiltered. We measured postoperative leucocyte counts and arterial blood oxygenation, and found that the postoperative leucocyte counts were significantly lower in the filter group than in the control group. This difference reached a maximum on the second postoperative day. However, in contrast to our study in adults, there was no difference in arterial blood oxygenation on the first postoperative day.

Chapter 4 addresses the question whether a leucocyte depletion filter removes activated granulocytes or a general leucocyte population. This has implications for the efficacy of the filtration process. After clinical use, we examined 11 filters morphologically and immunologically. In addition, β-glucuronidase was measured in 8 patients before and after the filter to determine whether leucocytes were activated during filtration. Microscopic evaluation revealed that granulocytes were trapped significantly more in the first blood contact layer of the filter material than in the other layers. A maximal CD45RO expression was measured on granulocytes trapped inside the filter material indicating that these granulocytes were activated. In contrast, the β-glucuronidase concentration did not increase after filtration, suggesting the absence of activation of granulocytes by the filtration process. These results suggest that a leucocyte depletion filter removes activated granulocytes rather than leucocytes at random and imply that a leucocyte depletion filter is suitable for use in cardiac surgical patients.

In chapter 5 three major leucocyte filtration strategies were compared in order to define optimal duration of the filtration procedure as well as flow and pressure conditions in the filter. These filtration strategies were: filtration of arterial blood throughout cardiopulmonary bypass (associated with high flow and pressure gradients), filtration of a part of the venous return blood in the rewarming phase during cardiopulmonary bypass (associated with intermediate flow, but high pressure), filtration of residual heart-lung machine blood during transfusion into the patient after cardiopulmonary bypass (associated with low flow and low pressure), and a control group without filtration. We measured circulating leucocyte counts, plasma elastase levels and arterial blood oxygenation, and examined filters postoperatively by scanning electronmicroscopy. Although we could not demonstrate a clinical difference among the three leucocyte depletion strategies, the laboratory results suggested that leucocyte filtration at low flow and pressure conditions is associated with less leucocyte damage and less release of elastase.

Chapter 6 gives an introduction into the concept of fat filtration. Recently, fat microemboli have been demonstrated in brain tissue after cardiopulmonary bypass. These were related to retransfusion of cardiotomy suction blood and associated with postoperative neurocognitive dysfunction. Therefore, attention is again focused on the adverse effects of retransfusion of cardiotomy suction blood during cardiac surgery. In addition, the role of fat on organ injury may have been underestimated, because fat microemboli have not only been demonstrated in brain tissue after cardiopulmonary bypass, but also in lung and renal tissue.

In chapter 7 the use of a fat removal filter for surgical wound suction blood was examined with emphasis on the efficacy of the filter in a clinical setting. We choose wound suction blood during cardiac surgery as this blood contains a considerable quantity of fat and particulate microemboli. Coronary artery bypass patients were randomly divided into two groups. In one group cardiotomy suction blood was filtered with a fat removal filter, in the other group this blood was retransfused without filtration. Filter efficacy was evaluated using biochemical assays and thin layer chromatography of blood samples taken simultaneously before and after the filter. In addition, clinical and biochemical markers for organ injury were determined in both groups. The fat filter removed 40% of fat, leucocytes and platelets from cardiotomy suction blood. Chromatography showed a significant reduction in free fatty acids,

phospholipids and triglycerides. Clinically, leucocyte counts were similar, but platelet counts were higher in the filter group on the first postoperative day. Although from this small scale study fat filtration appeared promising, a larger study is necessary to determine the clinical effects on organ injury.

In chapter 8 the fat filter was used for the cardiotomy suction blood during cardiac surgery, but emphasis was put on cerebral effects and on the inflammatory response. In one group of coronary artery bypass patients the cardiotomy suction blood was filtered with a fat removal filter and retransfused, in the other group the cardiotomy suction blood was completely discarded. We measured triglyceride and glycerol, and neuron-specific enolase and S-100β as brain injury markers, and circulating total white blood cell and granulocyte counts and interleukin-6 as inflammatory markers. Apart from a transient increase in S-100β and neuron specific enolase values in the filter group, there was no difference between the groups on the first postoperative day. Triglyceride levels on the first postoperative day were similar. Total white blood cell and granulocyte counts were higher in the filter group.

The filtration related transient increase in brain markers and the higher white blood cell and granulocyte counts in the filter group suggested that the filter efficacy should be improved.

Conclusions

In the clinical studies presented in this thesis leucocytes and fat particles were filtered during and after cardiac surgery. The results of these studies suggest a beneficial effect of perioperative leucocyte and fat filtration on postoperative tissue injury. The best clinical results were achieved with the filtration of the residual heart-lung machine blood. Filtration techniques for cardiotomy suction blood are promising. However, it should be kept in mind that the fat filter we used was not very effective. Therefore, with an improved filter better clinical results might be obtained.

There is evidence that leucocytes, fat and particulate all contribute to post-operative tissue injury. The results of this thesis suggest that leucocyte, particulate and fat filtration may be considered as one entity from an inflammatory point of view. Therefore, from a clinical point of view, one filter suitable for the cardiotomy suction blood as well as the residual heart-lung machine blood should be developed.

CHAPTER 10

SAMENVATTING EN CONCLUSIES

De hart-longmachine vormt door zijn grote contactoppervlak met het bloed van de patiënt een enorme prikkel voor stollings- en ontstekingsreacties. Deze ontstekingsreacties omvatten het gehele lichaam en leiden tot weefselbeschadiging die na de operatie gemeten kan worden. Leucocyten, ook wel witte bloedlichaampjes genaamd, en vooral granulocyten, die een subgroep vormen van de witte bloedlichaampjes, spelen een sleutelrol in die ontstekingsreacties. Om de postoperatieve weefselbeschadiging te verminderen of misschien zelfs te voorkomen hebben we de aandacht gericht op de leucocyten. We wilden hun activering zoveel mogelijk beperken. Verschillende methoden zijn hiervoor door anderen al onderzocht, zoals het aanbrengen van een coating op het contactoppervlak van de hart-longmachine, het gebruik van een cell-saver om wondzuigbloed dat doorgaans weer teruggegeven wordt aan de patiënt, schoon te wassen, het terugbrengen van het aantal vrije zuurstofradicalen en het gebruik van farmacologische middelen als aprotinine en corticosteroiden. Echter, uit dierexperimenten bleek een andere methode, het verwijderen van de leucocyten met behulp van een filter, het meest effectief te zijn.

Niet alleen geactiveerde leucocyten, maar ook vet en andere deeltjes die tijdens de operatie in de bloedbaan komen zoals luchtbelletjes, plastic van de hart-longmachine en kleine bloedstolsels, vormen een oorzaak van postoperatieve weefselbeschadiging. Kleine vetdeeltjes zijn na hartchirurgie in het hersenweefsel aangetoond en worden in verband gebracht met het functieverlies dat bij ongeveer een kwart van de patiënten drie maanden na de operatie nog bestaat. Deze vetdeeltjes zijn ook aangetoond in long- en nierweefsel. Door verbeterde filtertechnologie kunnen nu naast de leucocyten ook vetdeeltjes uit het bloed verwijderd worden.

Het doel van dit proefschrift is, zoals in hoofdstuk 1 is beschreven, aan te tonen dat het wegfilteren van leucocyten en vetdeeltjes tijdens hartchirurgie leidt tot afname van ontstekingsparameters en tot vermindering van de postoperatieve weefselbeschadiging.

In hoofdstuk 2 wordt een nieuwe, verbeterde methode beschreven om leucocyten te verwijderen. Tot dan werden tijdens een hartoperatie leucocyten verwijderd door een filter dat in de toevoerlijn van de hart-longmachine naar de patiënt was geplaatst. De resultaten daarvan waren niet eenduidig. Deze nieuwe methode richtte zich op het restbloed dat in de hart-longmachine overblijft aan het einde van de extracorporele circulatie en weer wordt teruggegeven aan de patiënt. Alleen dat bloed werd nu gefilterd. Wij onderzochten in een gerandomiseerde prospectieve studie bij 30 patiënten of deze nieuwe methode tot een verbetering van de longfunctie en een afname van de postoperatieve ontstekingsreactie zou leiden. In de filtergroep werden vóór teruggave aan de patiënt de leucocyten met een filter uit het restbloed van de hart-longmachine verwijderd. In de controlegroep werd een zelfde hoeveelheid rest-bloed ongefilterd teruggegeven. De patiënten in de filtergroep hadden minder leuco-cyten en granulocyten in hun bloed. Daarnaast hadden zij een uur na aankomst op de intensive care en na het ontwennen van de beademing een significant hogere zuurstof-spanning in het bloed. Deze resultaten duiden erop dat het wegfilteren van de witte bloedlichaampjes uit het restbloed van de hart-longmachine postoperatief inderdaad leidt tot een verbetering van de gaswisseling in de longen en tot een afname van de ontstekingsreactie.

In hoofdstuk 3 wordt een gelijksoortige studie beschreven bij kinderen die een hartoperatie ondergingen voor de correctie van een aangeboren hartafwijking. Bij

kinderen is de ontstekingsreactie bij gebruik van de hart-longmachine meer uitgesproken dan bij volwassenen. Dat komt in de eerste plaats omdat bij kinderen het contactoppervlak van het bloed met het inwendige van de hart-longmachine relatief groter is dan bij volwassenen. Daar komt nog bij dat kinderen met een cyanotische hartafwijking gevoeliger zijn voor de effecten van vrije zuurstofradicalen die ook bijdragen aan de ontstekingsreactie. We verwachtten daarom bij de kinderen een meer uitgesproken klinisch effect van het filteren van het restbloed uit de hart-longmachine dan bij de volwassenen. Bij 25 kinderen verwijderden we met een filter de leucocyten uit het restbloed van de hart-longmachine voordat we het bloed weer teruggaven. De resultaten vergeleken we met een controlegroep van 25 andere kinderen die dit restbloed ongefilterd terugkregen. We vonden dat het aantal leucocyten in de filtergroep significant lager was dan in de controlegroep. Het verschil was, in tegenstelling tot de bevindingen bij de volwassenen de tweede postoperatieve dag, maximaal. We konden echter, in tegenstelling tot de bevindingen bij de volwassenen, op de eerste postoperatieve dag geen verschil waarnemen in de arteriële zuurstof-spanning in het bloed. Onze verwachting, dat het klinische effect van bloedfiltratie groter zou zijn bij kinderen, werd dus slechts gedeeltelijk bevestigd.

In hoofdstuk 4 gaan we in op de vraag of een filter alle leucocyten verwijdert of alleen de geactiveerde. Dat zou gevolgen hebben voor de wijze waarop men een filter zou moeten inzetten. We onderzochten hiertoe 11 commercieel verkrijgbare filters. Na gebruik vonden we significant meer granulocyten in de eerste laag van het filtermateriaal dat met het bloed contact maakt dan in de volgende lagen. Immunologisch onderzoek toonde aan dat de expressie van het adhesiemolecuul CD45RO op de celmembraan van de granulocyten, die in het filter zaten, maximaal was. Dat betekent dat deze granulocyten geactiveerd waren. Daar staat tegenover dat de concentratie van het β-glucuronidase die gemeten was vóór en na het filter, niet verschilde. Dat duidt erop dat het filtermateriaal zelf de granulocyten niet activeerde. Deze resultaten suggereren dat een leucocytenfilter inderdaad alleen de geactiveerde granulocyten wegvangt, hetgeen betekent dat toepassing van een leucocytenfilter nuttig kan zijn om een ontstekingsreactie te verminderen.

Om inzicht te krijgen in de optimale duur van de filterprocedure en de effecten van de bloedstroom en de drukverhoudingen in het filter, vergeleken we in hoofdstuk 5 drie methoden om leucocyten weg te filteren. Die methoden zijn: (1) het filteren van bloed in de arteriële toevoerlijn van de hart-longmachine (met hoge bloedstroom en hoge drukgradiënt), (2) het filteren van een deel van het bloed uit de veneuze afvoerlijn van de hart-longmachine (met middelmatige bloedstroom en hoge druk-gradiënt) en (3) het filteren van het restbloed uit de hart-longmachine na extra-corporele circulatie (met lage bloedstroom en lage drukgradiënt). In een vierde patiëntengroep werd geen filter toegepast. Dit was de controlegroep. We vonden geen verschil in klinische uitkomst tussen de drie methoden. Wel duidde laboratorium-onderzoek erop dat het filteren van leucocyten bij lage bloedstroom en lage druk de minste schade veroorzaakte aan de leucocyten.

In hoofdstuk 6 worden als inleiding tot de volgende hoofdstukken enkele aspecten van het filteren van vetdeeltjes besproken. Vetten lossen slecht op in bloed en vormen daar deeltjes. Na hartchirurgie waarbij de hart-longmachine werd gebruikt, zijn dergelijke vetdeeltjes aangetoond in onder andere de hersenen, longen en nieren van patiënten. Deze vetdeeltjes worden rechtstreeks in verband gebracht met de

beschadiging in functie die bij veel patiënten na hartchirurgie aantoonbaar is. Men vond deze vetdeeltjes ook terug in het wondzuigbloed dat tijdens de operatie veelal direct weer wordt teruggeven aan de patiënt. Hiermee is de aandacht gevestigd op een nadelig effect van het teruggeven van het wondzuigbloed tijdens de operatie.

In hoofdstuk 7 onderzochten we bij coronaire bypass operaties de effectiviteit van een vetfilter voor het chirurgische wondzuigbloed. Dat vetfilter was afgeleid van een leucocytenfilter. In de filtergroep werd het wondzuigbloed vóór teruggave aan de patiënt gefilterd. De resultaten werden vergeleken met een controlegroep waarin het wondzuigbloed ongefilterd werd teruggegeven. De effectiviteit van het filter werd beoordeeld aan de hand van chromatografie en van biochemische bepalingen van bloedmonsters die tegelijkertijd vóór en na het filter werden afgenomen. Het vetfilter verwijderde 40% van het vet, maar ook 40% van de leucocyten en bloedplaatjes uit het wondzuigbloed. In de bloedmonsters na het filter werd met chromatografie eveneens een significante afname gevonden van de vrije vetzuren, de fosfolipiden en de triglyceriden. Het aantal leucocyten in het bloed was in beide groepen niet verschillend op de eerste postoperatieve dag. Wel was het aantal bloedplaatjes in de filtergroep hoger. Deze resultaten tonen aan dat het filteren van vetten in de kliniek mogelijk is, en dat er naast vetten ook andere belangrijke bloedelementen tegelijkertijd verdwijnen. De effectiviteit van het vetfilter lijkt echter voor verbetering vatbaar.

In hoofdstuk 8 beschrijven we bij patiënten, die een coronaire bypass operatie ondergingen, enkele effecten van het gebruik van het vetfilter voor het wondzuigbloed, op de hersenen en op de postoperatieve ontstekingsreactie. In de filtergroep werd het wondzuigbloed vóór teruggave aan de patiënt gefilterd. In de controlegroep werd het wondzuigbloed helemaal niet meer teruggegeven. Als indicator voor schade aan de hersenen bepaalden we de bloedspiegels van de enzymen neuron specifieke enolase en S-100β. Als indicator voor de mate van de ontstekingsreactie bepaalden we de hoeveelheid leucocyten en granulocyten in het bloed alsmede de cytokine Interleukine-6. Afgezien van een kortdurende toename in het bloed van de enzymen neuron specifieke enolase en S-100β vonden we op de eerste postoperatieve dag geen verschillen tussen beide groepen. De aantallen leucocyten en granulocyten in het bloed van de filtergroep waren echter hoger dan in de controlegroep, waaruit we concludeerden dat de effectiviteit en de capaciteit van het filter zeer beperkt waren.

Conclusies

In de klinische studies, die beschreven zijn in dit proefschrift, werden leucocyten en vetdeeltjes op verschillende wijze tijdens of na een hartoperatie, uit het bloed gefilterd. De resultaten van die studies duiden er op dat perioperatieve filtratie van bloed inderdaad een gunstig effect heeft op de weefselbeschadiging en op de ontstekingsreacties na een hartoperatie. De beste klinische resultaten met betrekking tot weefselbeschadiging en ontstekingsreacties werden behaald met de techniek waarbij het restbloed van de hart-longmachine werd gefilterd. Verbeteringen aan het vetfilter zullen enkele nadelige effecten van de huidige filters verder moeten doen verminderen. Uit praktische overwegingen zou gestreefd moeten worden naar het ontwikkelen van één type filter, waarmee zowel het wondzuigbloed als ook het restbloed van de hart-longmachine kan worden gefilterd.

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CURRICULUM VITAE

De auteur van dit proefschrift doorliep het gymnasium in Assen en ‘s-Hertogenbosch. Na het behalen van het diploma begon hij in 1972 rechten te studeren in Groningen. In 1975 behaalde hij het kandidaatsexamen. Na zijn militaire diensttijd begon hij met de studie geneeskunde. In 1984 behaalde hij het artsdiploma. Als student had hij gedurende enkele jaren assistentschappen bij de afdeling revalidatie. Dit vormde onder prof. dr. L.N.H. Goëken een eerste aanzet tot een wetenschappelijke studie. In 1986 begon hij met de opleiding anesthesiologie in Groningen (opleider prof. dr. D. Langrehr). Na zijn opleiding is hij vanaf 1990 steeds werkzaam geweest binnen de sectie thoraxanesthesiologie van het Academisch Ziekenhuis Groningen.

STELLINGEN BEHORENDE BIJ HET PROEFSCHRIFT

CLINICAL ASPECTS OF LEUCOCYTE AND FAT FILTRATION DURING CARDIAC SURGERY.

1. Het verdient aanbeveling een celsaver met een leucocytenfilter uit te rusten.

2. Dat de klinische resultaten van leucocytenfiltratie soms te wensen overlaten heeft te maken met het feit dat het niet duidelijk is hoeveel leucocyten precies verwijderd moeten worden.

3. Gezien het toenemende gebruik van bloedplaatjesremmende medicatie bij patiënten die hartchirurgie ondergaan dient recombinant factor 7a eerder gebruikt te worden bij postoperatief bloedverlies dan nu het geval is.

4. Voor een succesvolle toepassing van autologe retransfusiesystemen voor wondbloed dienen deze met betere filters te worden uitgerust.

5. Gezien haar gunstige postoperatieve effecten verdient epidurale analgesie een ruimere toepassing binnen de hartchirurgie.

6. Het tekort aan donoren berust er voor een groot deel op dat toestemming voor het afstaan van organen voor transplantatie te weinig wordt gevraagd én verkregen.

7. Ter vermindering van reperfusieletsel dient men getransplanteerde organen eerst met leucocytenvrij bloed te perfunderen.

8. Goede patiëntenzorg krijgt meer betekenis door een geïntegreerd patiënten datamanagementsysteem.

9. Een godsdienst die de maatschappelijke veranderingen in de positie van de vrouw ontkent verliest zijn mondiale betekenis.

10. Een goed chirurg verdient een goede anesthesist, een slecht chirurg heeft er zeker één nodig.

11. Rust als enige therapie is ziekmakend.